Surgical castration in piglets: its impacts on pain and affective states

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Master of Science

In

Animal and Poultry Sciences

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October 24th, 2022

Blacksburg, Virginia

Keywords: animal welfare, surgical castration, piglet, pain assessment, questionnaire; swine industry

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ABSTRACT

Surgical castration of young male piglets is an extremely common husbandry procedure in the United States swine industry, with nearly 100% of males castrated. The procedure is performed primarily to prevent the occurrence of boar taint in the meat and to reduce aggression as the animals age. No research has investigated the impact of surgical castration on the long-term mood states – also known as the affective state – of piglets, but it has been proven to cause both acute and chronic pain that can last up to 4 days, modify piglet behavior, and reduce weight gain. Because of this, feasible approaches for pain alleviation are needed. A hinderance to this is that currently no analgesics are approved for piglets. In addition, we need validated, practical methods to recognize pain in piglets in order to help researchers, veterinarians, and caretakers to identify the need for analgesics. One useful tool for this could be the Piglet Grimace Scale, which has previously been validated in this context, but is not widely applied. The current lack of analgesic use during castration may be cause for concern for consumers, however little is known about the public's knowledge on the industry's practices. Therefore, the thesis' objectives are to (1) assess the impact of surgical castration on pain and affective states, (2) determine people's ability to recognize pain expression after surgical castration, and (3) get insights into public perception of swine industry practices. In Chapter 3, we assessed the impact of surgical castration and practical pain alleviation methods during and after castration on piglets' short and long-term affective states and activity levels. Piglets were surgically castrated, castrated with analgesics, or sham-handled at 3 days of

age. Data on piglets' activity (using accelerometers), tails (affective states), and grimaces (pain expression) were collected for 1, 6, and 24 hours after castration treatments while piglets were in their home pens. An attention bias test was then performed in week 1 and 12 to assess anxiety (an affective state). Home pen activity after castration treatments suggested that there was a positive impact of using analgesics. The piglets' behavior (eating) and activity during the attention bias test at week 1 suggest a negative impact of castration on affective states. The differences observed in week 1 were no longer found in week 12, suggesting that any impact of surgical castration at 3 days of age on affective states are no longer detectable at 12 weeks of age. In Chapter 4, through an online survey we investigated the validity and reliability of Piglet Grimace Scale ratings applied by swine industry professionals and the general public, to assess its potential utility in non-research settings. The survey contained a training, followed by 9 piglet images showing facial expressions immediately after castration or sham-handling. Both response groups were able to recognize pain in castrated piglets. However, both response groups overestimated pain experience compared to trained experts, suggesting more training may be necessary. Nevertheless, overall, the scale can be widely applied by veterinarians, industry professionals and even members of the public. In Chapter 5, a survey provided initial insights into public knowledge and perceptions on castration and analgesia use and compared this to industry stakeholders. The results show that knowledge on industry practices was especially lacking for public respondents, but also for a minority of industry respondents, indicating opportunities for education and further research on the topic.

Surgical castration in piglets: its impacts on pain and affective states

Jessica Michelle Neary

GENERAL AUDIENCE ABSTRACT

Surgical castration is performed on nearly every male piglet in the United States swine industry. Males are castrated to prevent the occurrence of boar taint and to reduce aggression. Boar taint is the unpleasant odor produced by the skatole and androsterone in the fat when the meat is prepared. It is unclear how this practice impacts piglet's long-term mood states (affective states), but we do know it causes pain, changes in behavior, and reduced productivity. Thus, we need feasible approaches for pain relief. However, there are currently no pain relievers approved for piglets. In addition, we need validated, practical methods to quantify pain in piglets in order to identify the need for pain relief. One useful tool is the Piglet Grimace Scale, which is validated but is not widely applied. The current lack of pain relief use during castration may concern consumers, yet no data is collected on this. Therefore, the thesis' objectives are to (1) assess the impact of surgical castration on pain and affective states, (2) determine people's ability to recognize pain expression after surgical castration, and (3) get insights into public perception of swine industry practices.

In Chapter 3, we assessed the impact of surgical castration and practical pain alleviation methods during and after castration on piglets' short and long-term affective states and activity levels. Piglets were surgically castrated, castrated with analgesics, or sham-handled at 3 days of age. Home pen activity after castration treatments showed a positive impact of using analgesics. The piglets' behavior and activity during an attention bias test at week 1 suggest that piglets were experiencing a negative emotional state after

castration. The differences seen in week 1 were no longer found in week 12. This suggests that any potential impact of surgical castration on affective states are no longer detectable at 12 weeks of age. In Chapter 4, an online survey was used to investigate the Piglet Grimace Scale ratings applied by swine industry professionals and the general public. After training, respondents scored 9 images showing facial expressions of piglets after castration or sham-handling. Both public and industry people were able to recognize pain in castrated piglets. However, both response groups overestimated the pain experience compared to trained experts, suggesting more training may be necessary. Nevertheless, overall findings suggest that the scale can be widely applied. In Chapter 5, a survey provided insights into public knowledge and perceptions on castration and pain relief use in the industry, and compared this to industry stakeholders. The results showed that knowledge on industry practices was especially lacking for public respondents, but also for a minority of industry respondents, indicating opportunities for education and further research on the topic.

Acknowledgements

First and foremost, I'd like to thank my research advisor and committee chair, Dr. Leonie Jacobs. As a non-typical graduate student, I'm grateful for the continued guidance and patience throughout my masters' journey – whether it's experimental designs, writing manuscripts, or learning statistics. You've taught me so much during my time at Virginia Tech and have given me a new perspective on things. Through your words of advice and continued support, you've given me the opportunity to grow, not only as a scientist but as a person, and I cannot thank you enough for all of it.

I'd also like to thank my committee members, Dr. Gareth Arnott and Dr. Sherrie Clark-Deener. You have both been amazing examples for me and I'm truly grateful for having the opportunity to work with you both. Gareth, I'm grateful for the email exchanges and the support and encouragement you provide. Sherrie, I aspire to be half the teacher and person you are. Your presence, encouragement, and guidance are what I hold dear and I cannot express how appreciative I am of everything that you've done for me.

Drew, Gracie, Bidur, Ally, and Marconi, thank you for always being ready to share a laugh and deal with the fact that I'm probably going to smell like pigs during lab meetings. I cannot wait to see all of the amazing things you guys are going to accomplish. Jordyn, Lexie, and Sydney – I don't know what I would do without you guys. You have always been in my corner when I needed you, and I'm forever grateful to have you in my life.

Emily, Jake, Garrett, and Chris – I want to thank you for all the encouragement and support. Whenever I needed a laugh or a break from graduate school, there was always something going on at the farms that could provide a much-needed distraction. Sadie, Dana, Edythe, and all the undergraduates that worked at the Swine Center – your dedication and work ethic are inspiring. I could not have accomplished what I have in the last 3 years without you all and for that I'm truly grateful.

Dad, thank you for showing me the value of a hard days' work and how rewarding working with livestock can be. Mom, thank you for always being available to listen and give advice, pushing me to succeed, and teaching me to do everything with pride and passion. James and Janella, I'm lucky to be your older sister. The four of you are a core part of my life – I love you more than I could ever put into words. I wouldn't be where I am or who I am without your constant love, encouragement, and support.

I cannot thank the people in the School of Animal Sciences enough. I'm forever grateful to be a part of this community and to have had the opportunity to learn from you, teach students with you, and grow as a person.

Finally, I would like to thank the Virginia Pork Council for their financial support of the experiment described in Chapter 4. Furthermore, I would like to thank the USDA National Institute of Food and Agriculture, (Hatch Multistate project 1024623) for support of the work outlined in Chapter 5 and 6.

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Chapter 1: Introduction

The study of animal welfare has been a rapidly growing field of scientific study for the last 30 years (Broom 2011). Many people considered welfare a key factor when determining if a system involving animals is sustainable (Broom 2019). Broom (1986) defined welfare as the states of an individual in regards to how it attempts to cope with its environment (Broom 1986). Carenzi and Verga (2009) have more recently stated that the broadest definition of animal welfare needs to include the comprehensive state of animal, meaning the body and mind need to be considered along with everything in between (Carenzi & Verga 2009). However, the term needs a strict definition when being used in science, legal documents, and public discussions (Broom 2019). The concept of the Five Freedoms (Table 1.1) are typically referenced when animal welfare appears in these contexts (Mellor 2016, Webster 2016). However, this has been criticized in recent years because the Five Freedoms mainly focus on negative experiences and focus more on moments in time rather than taking a wholistic view (Mellor 2016, Webster 2016). The Five Freedoms do have value in the fact that they can help sculpt management practices that help keep animals alive, but the animals are not necessarily thriving. Animal welfare research continues to grow and develop as a science. To accomplish this, there is the opportunity to build upon the established concepts of avoiding negative emotions (or "negative welfare") and consider the assurance of positive emotions ("positive welfare"), where the animal has a "life worth living" (Mellor 2016, Webster 2016).

Table 1.1 The Five Freedoms and Provisions. Adapted from Brambell (1965) and FAVC (1993).

Freedom	Ensured by providing
From thirst, hunger and malnutrition	Ready access to fresh water and a diet to maintain full health and vigor
From discomfort and exposure	An appropriate environment, including shelter and a comfortable resting area
From pain, injury, and disease	Prevention or rapid diagnosis and treatment
From fear and distress	Ensuring conditions and treatment which avoid mental suffering
To express normal behavior	Sufficient space, proper facilities, and company of the animal's own kind

A more comprehensive idea of animal welfare involves three overlapping concepts: affective state, biological function, and natural living (Figure 1.1) (Fraser 2008). It is important to consider the connectivity of these ideas when considering approaches in evaluating animal welfare because a single measure does not cover all dimensions of welfare (Botreau et al. 2007). The affective state component focuses on an animal's emotions and feelings (affective experiences). These can be positive, negative, or neutral. For an animal to have good welfare regarding this component, it should be free of negative emotions, like pain and suffering, and be experiencing positive emotions like pleasure and play. The second component, natural living, focuses on an animal's ability to do as it would in the wild. Good welfare in this sense would allow for the animal to express natural behaviors. Finally, the concept of biological functioning focuses on aspects of good health like nutrition, growth, and reproduction. An example of good welfare in this aspect would be when an animal is receiving proper nutrition and able to maintain a good body condition score. All three of these concepts need to be taken into consideration when evaluating animal welfare.

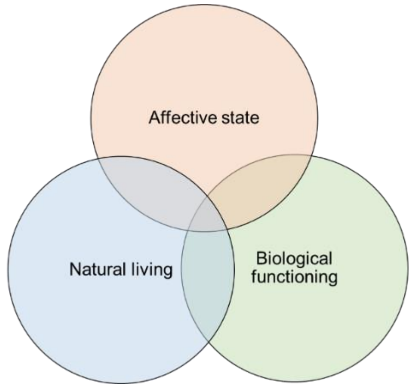

Figure 1.1. Three concepts of animal welfare. Adapted from (Fraser 2008).

Emotions can be defined as short-term states produced by stimuli that cause an animal to work to either gain or avoid said stimuli, which in turn help the animal to respond to changes around them (Crump et al. 2020). Affective states can be defined as a long-lasting mood state resulting from an accumulation of subjective experiences and emotions, and can range between negative and positive in valence (Mellor 2014). Animals adapted emotionally to respond to stimuli in their environment and this plays a large role in forming an animal's affective state (Mendl et al. 2010, Crump et al. 2020). Meaning, if an animal is surrounded by stimuli that cause it to constantly experience distress and prevent it from engaging in preferred activities, then this will lead to negative emotions and the animal will be in an overall negative affective state. Likewise, if an animal is surrounded by stimuli that allow it to express highly-motivated, species-specific behaviors, then this will lead to positive emotions and an overall positive affective state (Mendl et al. 2010). Assessing affective state is an integral part of assessing overall animal welfare because it provides information on how the animal perceives its environment.

In the United States, pork production is one of the largest components of the animal agriculture industry. According to the U.S. Department of Agriculture (USDA), 27.7 million pounds of pork was produced in 2019, valued at 13.28 million dollars (USDA ERS 2022). Most pigs raised in the

United States are done so in some sort of confinement production that allows for year-round production and protects pigs from things like disease and predators (USDA ERS 2021). There are several stages of the pig industry, with the first one being farrow-to-wean. While the piglet is still with the sow, it will undergo several neonatal care procedures which include tail docking, vaccinations, and iron injections. Another step that goes along with these is the surgical castration of male piglets. Surgical castration involves manually restraining the boar, making two incisions on the scrotum, removing the testicles, and cutting or tearing the spermatic cord (AVMA 2013). Although there are other factors, the biggest reason for castration is to prevent the development of boar taint, which is a foul odor when the meat it prepared caused by the post-pubertal deposition of skatole and androsterone in the animal's body fat (Keenan 2016). Castration has been shown to impact piglets' physiology and behavior, meaning it impacts their biological functioning as well as their ability to perform natural behaviors. To fully encompass the three components of animal welfare, the impact of castration on piglets' affective state needs to be investigated.

This thesis evaluates our current understanding of surgical castration and its impact on affective state in piglets. The following literature review (Chapter 2) focuses on current castration practices in the United States and surgical castration's impact on piglets, affective states, attention bias tests, and pain recognition tools in pigs. Following that, there are three chapters that have all been submitted for publication in peer-reviewed journals. Chapter 3 investigates the short- and long-term impacts on surgical castration in piglets. Chapter 4 evaluates industry professionals and laypeople's ability to recognize pain in piglets using a grimace scale. Chapter 5 evaluates industry professionals and laypeople's knowledge of the swine industry's castration practices. Finally, Chapter 6 consists of a general discussion and conclusion.

Chapter 2: Literature Review

2.1 United States Swine Production

The United States (U.S.) is one of the world's largest producers, consumers, and exporters of pork and pork products (USDA ERS 2019). According to the U.S. Department of Agriculture (USDA), 125.5 million market hogs were slaughtered in 2021 with an average price of $67.28 cwt (per hundred pounds) (USDA ERS 2022). The structure of the industry has shifted dramatically over the last 40 years. Since 1990, the number of farms has declined by more than 70%, as individual enterprises were integrated (USDA ERS 2021). In general, U.S. swine production consists of 4 stages: farrow-to-wean, nursery, grow-finish, and the processing plant once they reach approximately 127 kg (280 lbs; Figure 2.1).

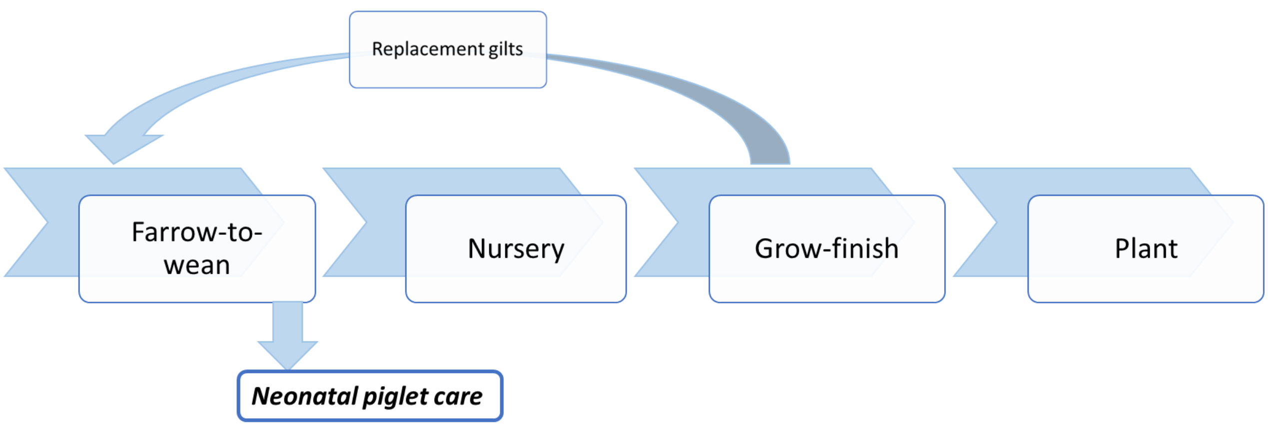

Figure 2.1 Typical production flow in the U.S. commercial swine industry. The focus of this thesis is on neonatal piglet care that takes place in the farrow-to-wean phase.

2.1.1 Farrow to Wean

In the farrow-to wean phase, piglets will typically spend 3 weeks on the sow farm prior to weaning. Within those 3 weeks, all piglets will undergo several neonatal care procedures, which include tail docking, vaccinations, and iron injections. Another step that goes along with these is the surgical castration of male piglets. When performing surgical castration, one manually restrains the boar, makes two incisions on the scrotum, manual removes the testicles, and cuts or tears the spermatic cord (AVMA 2013). In this thesis, the focus lies on the surgical castration of male piglets during the farrow-to-wean and nursery phases (Figure 2.1).

2.1.2 Castration in the United States and around the world

Castration of male piglets is performed for two reasons. One is to prevent aggressive behaviors towards caretakers and pen mates as boars reach reproductive age (Rydhmer et al. 2006). However, the main reason for castrating boars is to prevent the development of boar taint, which is a foul odor when the meat is prepared caused by the post-pubertal deposition of skatole and androsterone in body fat (Keenan 2016). According to the U.S. Food Safety and Inspection Service (FSIS), carcasses that give off a pronounced boar taint should be condemned (9 CFR 311.20 - Sexual odor of swine. 2012). If boar taint is less pronounced, the carcass may be included comminuted cooked meat products or for rendering. To avoid any financial consequences of boar taint and carcass quality issues, U.S. producers castrate all males destined for market (Rault et al. 2011).

There have been shifts away from surgical castration in other countries. In 2010, European swine industry stakeholders and non-governmental animal welfare organizations agreed upon the ‘European Declaration on alternatives to surgical castration of pigs’ and consisted of two levels to phase out surgical castration (European Union 2016). The first level intended for all surgical castration in Europe to be performed with prolonged analgesia and/or anesthesia by January 1, 2012. The second level was that surgical castration would be phased of out of all European Union countries and all European Free Trade Association countries by January 1, 2018. In a 2016 survey of the EU nations, 18 of the 24 countries surveyed indicated that ≥80% of all males were surgically castrated. Of those males, 5% were surgically castrated with anesthesia and analgesia, and 41% with analgesia alone (de Briyne et al. 2016). Ireland, Portugal, Spain, the Netherlands and United Kingdom reported surgical castration was used in ≤20% of males. In those countries, males are slaughtered at a lighter, prepuberal weight, thus avoiding the potential for boar taint to develop and dramatically decreasing the need for surgical castration (de Briyne et al. 2016). Surgical castration is still widely used in the U.S. and Brazil along with several countries in the EU (de Briyne et al. 2016, Hötzel et al. 2020). In contrast, immunocastration has been the only form of castration used in New Zealand and Australia since 1998 (American Veterinary Medical Association Welfare Division 2013).

2.1.3 Welfare Concerns Associated with Surgical Castration

Surgical castration in the U.S. is performed in the first week of age without analgesics or anesthetics. The American Veterinary Medical Association (AVMA) recommends that after day 14 of age, boars should be castrated using analgesia, anesthesia, or both (AVMA 2013). Even though the AVMA does not require any type of analgesia or anesthesia at the age that most commercial piglets are surgically castrated, research indicated that the procedure is painful and therefore a welfare concern. Surgical castration without analgesia or anesthetics will result in both physiological and behavioral responses indicative of pain. Behavioral impacts of surgical castration without analgesia include trembling, tail wagging, isolation, and increased vocalizations, and are summarized in Table 2.1. Physiological impacts of surgical castration without analgesia in piglets include increased levels of adrenocorticotropin hormone, cortisol, and lactate along with increased body temperatures, and are summarized in Table 2.2.

Table 2.1 Summary of research that reported behavioral indicators of pain in piglets that were surgical castrated without analgesics. - = decreased frequency + = increased frequency; n/a = not applicable

Reference	N	Age of castration	Responses and measurement methods	Response hours after castration	Response days after castration
(Hay et al. 2003)	84	5 days	Scan sampling during day and continuous sampling at night	- Activity at the udder + Inactivity while awake, prostration, stiffness, trembling, rump scratching, tail wagging, huddling, walking, self-isolation	+ Scratching the rump, tail wagging, huddling, desynchronization
(Taylor et al. 2001)	84	3, 10, or 17 days	Vocalizations recorded during castrations; continuous behavioral observation	+ High-frequency and low-frequency calls, sitting and standing	- Lying down, older piglets missed more nursings + Time at the udder
(Sutherland et al. 2010)	40	3 days	Vocalizations recorded during castrations; behavioral scan sampling	+ Stress vocalizations, isolation	n/a
(Gottardo et al. 2016)	198	4 days	Behavioral scan sampling, facial expression, and scrotal skin pressure sensitivity	+ Scrotal sensitivity, tremors, rubbing the scrotum area on the floor, hunching the back, wagging of the tail, isolation	n/a

Table 2.2 Summary of research that reported physiological indicators of pain in piglets that were surgical castration without analgesics. + = increased frequency

Reference	N	Age of castration	Responses and measurement methods	Response after castration
(Prunier et al. 2005)	126	7 days	Plasma cortisol, adrenocorticotropin hormone, glucose and lactate concentrations in the blood	+ adrenocorticotropin hormone, cortisol, and lactate
(Sutherland et al. 2010)	40	3 days	Blood samples were collected from piglets before and 30, 60, 120 and 180 min after castration to measure leukocyte and differential counts and cortisol concentrations	+ Cortisol concentrations at 30 and 60 min after castration
(Lonardi et al. 2015)	24-32 depending on response variable	4 days	cortisol, lactate, glycaemia, rectal and eye temperature	+ Cortisol and a positive correlation with glucose concentration, eye temperature rectal temperature
(Gottardo et al. 2016)	198	4 days	serum cortisol levels at different timepoints within 300 minutes of castration	+ serum cortisol concentration at 60 minutes after

2.1.4 Alternatives to Surgical Castration

While surgical castration without analgesics is one of the most common methods of castration in the industry, there are several alternatives available. Immunocastration and castration with analgesics are two that show good feasibility. Improvest® is an immunocastration product that was approved by the FDA in 2011 and requires a prescription from a veterinarian. It involves a protein compound that induces antibody production against gonadotropin releasing hormone (GnRH). This immunization decreases the production of androsterone and skatole, which are responsible for boar taint. Boars receive two injections of Improvest® during its life which induce the production of antibodies that stop the production of androsterone and skatole (AVMA 2013). The first dose is given any time after the animal is 9 weeks old and acts as a primer. The second dose needs to be administered 4 to 8 weeks after the first dose. This process is less painful than surgical castration, however there are some welfare implications that need to be considered. Anecdotal observations showed that for immunocastration some boars may not properly receive both injections. The animal will not produce the necessary antibodies if this occurs. The boar will continue to show behaviors associated with intact males and could even develop boar taint. There is also the chance that a boar will have a negative reaction at the injection site. Along with that, there is the risk of a needle breaking off during administration. It has been argued that Improvest® lacks wide adoption in the U.S. because of a lack of awareness among producers, however it is widely used in other pork-producing nations (Rueff et al. 2019).

Another solution to resolve some of the welfare concerns associated with surgical castration is applying analgesics during the procedure to help mitigate pain. However, farmers face legal and financial restraints when considering analgesics (Steagall et al. 2021). In the U.S., no pharmaceuticals are approved by the U.S. Food and Drug Administration (FDA) for use for pain in pigs (Bates et al. 2014). A veterinarian could prescribe pharmaceuticals, but only ones that are regulated under the Animal Medicinal Drug Use Clarification Act of 1994 to mitigate pain (AMDUCA 1994). In order for an analgesic to gain approval through the FDA, methods of pain assessment and drug efficiency need to be well-defined and validated and further work is needed to accomplish this (Wagner et al. 2020). Yet, veterinarians could consider prescribing extra-label use of non-approved analgesics. From an economic standpoint, producers have to consider not only the cost of purchase, but also the costs of labor and supplies to administer analgesics. It is difficult for veterinarians to make strong, economically grounded arguments for implementing pain mitigation protocols when you consider the economic impact and lack of approved analgesics

(Coetzee 2011). Therefore, in current U.S. practice, no analgesia or anesthesia are used during or after surgical castration of boars.

Other alternatives to surgical castration and immunocastration exist. For example, raising entire males, sperm sexing for females, and injecting certain chemicals compounds into the testes including potassium permanganate with acetic acid, silver nitrate lactic acid, or zinc acetate. However, these are not viable for use within the industry on the short-term. These three options are summarized in Table 2.3.

Table 2.3 List of alternative approaches to surgical castration or immunocastration with their (dis)advantages, level of feasibility, and reference.

Alternative approach	Advantage(s)	Disadvantage(s)	Short-term feasibility	Reference(s)
Raising entire males	1. Avoids the labor and pain associated with surgical castration 2. Reduction of feed costs and impact on the environment due to a higher feed efficiency 3. Increase in muscle and unsaturated (healthy) fat	1. Difficulties with raising and housing entire male 2. Decreased welfare of those low in the hierarchy of a pen 3. Risk for penile injuries 4. Lower meat quality in relation to the reduced intramuscular fat content 5. More frequent occurrence of DFD meat 6. Increased fat unsaturation 7. Occurrence of boar taint	Yes with 1. management changes 2. housing changes 3. additional costs 4. consumer expectation shifts	(Bonneau & Weiler 2019, Larzul 2021, Weiler et al. 2021)
Sperm sexing to produce females only	1. Avoids castration of males 2. Avoids the difficulties and risks associated with working with males	1. Sexing sperm in pigs is tedious, inefficient and expensive	No because 1. cost 2. lack of efficient technology	(Prunier et al. 2006, Bonneau & Weiler 2019)
Injecting chemicals (potassium permanganate + acetic acid, silver nitrate lactic acid, or zinc acetate) into the testes to destroy tissue	1. Easy to administer 2. Safe for caretakers to administer 3. Inexpensive	1. Causes swelling and pain in the testes 2. Labor and chemical costs 3. Depending on the chemical, can be somewhat ineffective	No because 1. up-to-date research is lacking 2. pain is difficult to determine 3. few effective chemicals are approved for the procedure	(Prunier et al. 2006, Bonneau & Weiler 2019)

2.2 Affective state: Defining important terms

There are terms like "emotion", "mood" and "affective state" that are used in both human and non-human research. However, their definitions differ depending on the scientific literature (de Vere & Ii 2016, Kremer et al. 2020). These differences in interpretations of these terms has led to some blocks in progress within the field (Paul & Mendl 2018, Mendl & Paul 2020), and is part of the reason there is debate if any non-human species can experience emotion (de Vere & Ii 2016). The definition of emotion varies:

> "Certain neurophysiological states, inferred from behavior, about which little is known except that by definition the predispose toward certain specific kinds of action"(Hebb 1946)

> "A temporary state brought about by biologically relevant stimuli, marked by specific changes in the organism's body and mind" (de Waal 2011)

> "Short-term states elicited by stimuli (or their predictors) that animals will work to acquire (rewards, e.g., prey) or avoid (punishments, e.g., predators)" (Crump et al. 2020)

These three definitions are in order of how specific they are. The first one vaguely recognizes that emotions may influence an animal's actions but does not explain how or give any indication of a timeframe. The second definition is a bit more specific and suggests that emotions are generated from external stimuli that cause specific biological changes and behavioral reactions. The second definition also notes the short-term nature of emotions. This definition fails to include the outward expression of emotions. The third definition contains the most detail, describing how emotions are produced, their results, and their duration. Although these and all definitions of animal emotion vary within scientific literature, all have a general theme. All seem to have some component that indicate an animal emotion is a biologically comprehensive (in terms of behavior, physiology, and subjectively) and valanced (positive or negative) response that can differ in arousal and duration (Paul & Mendl 2018). In this thesis, we choose a definition of emotion modified from Crump et al. (2020): short-term functional states produced by stimuli that cause an animal to work to either gain or avoid said stimuli, which in turn help the animal to appropriately respond to environmental changes.

Defining mood has proven to be equally as challenging. Mood has been described as a prolonged state experienced without the presence of a person, condition, thing, or event (Russell 2003). This definition is lacking in the fact that there is no specification of duration of stability. More specifically, mood can be defined as the outcome of the collection of previous emotional experiences, resulting in a "running mean" of positions across the two-dimensional space of valence and arousal over time (Mendl et al. 2010, Nettle & Bateson 2012). Our definition of emotion noted that they are partially characterized by their short-term nature, mood can be defined as the long-term, diffuse state that reflects the cumulative valence (positive or negative) of emotions over time (Mendl et al. 2010). The terms mood and affective state have been used interchangeably (Crump et al. 2020). Affective states have also been described as an animal's long-term mood state combined with their short-term emotional responses to current events, like an animal that is in a negative long-term mood state could temporarily experience positive affect states after rewarding stimuli like food or mating success (Mendl et al. 2010). In this thesis, we use the definition of affective state as an umbrella term for mood since it is more technical (Mendl

& Paul 2020). We also avoid an anthropomorphic interpretation of long-term valanced states when using the affective state instead of mood (Mendl & Paul 2020).

Valence and arousal are the two core factors used to conceptualize emotion and affective state (Mendl et al. 2010, Kremer et al. 2020). Valence refers to the way an animal perceives a situation and can either be positive (rewarding or pleasant), negative (punishing or unpleasant) or somewhere in between (Mendl et al. 2010, Kremer et al. 2020). Arousal refers to the intensity of the emotion or state and indicates the importance or urgency of the stimulus for specific emotions (Crump et al. 2020). The interaction of valence and arousal are shown in Figure 2.2, where Q1 and Q2 would be considered positive affective states and Q3 and Q4 negative affective states (Mendl et al. 2010). Discrete emotions can be applied somewhere in this framework as well, but their general location per se does not fully capture their subjective qualities (Mendl et al. 2010). Q1 represents a high arousal, positively valanced state (e.g., "excited" or "happy") (Mendl et al. 2010, Crump et al. 2020). Animals in this affective state function in a reward acquisition system, meaning they will actively work to acquire a reward (Rolls 2000, Mendl et al. 2010, Nettle & Bateson 2012, Mendl & Paul 2020). An example could be a predator that successfully captured prey, leading to a state of excitement as the predator eats. Q2 represents a positive, low arousal affective state, which can be associated with terms like "calm" or "relaxed" (Mendl et al. 2010). An animal in this state is usually in an environment where it's needs are met and there are few threatening stimuli (Mendl et al. 2010). Q3 represents negative, low arousal affective states, can be described with terms like "sadness" and "depression", and reflect an animal that has lost a reward or were certain resources may be limited (Mendl et al. 2010, Nettle & Bateson 2012, Mendl & Paul 2020). Finally, Q4 represents a negative, high arousal state that are associated with terms like "fearful" or "anxious" and the can lead to the behavioral responses to the threatening stimulus like vigilance (Mendl et al. 2010, Nettle & Bateson 2012, Crump et al. 2020). An example of this may be a prey species that has had encountered a predator before and senses one around again.

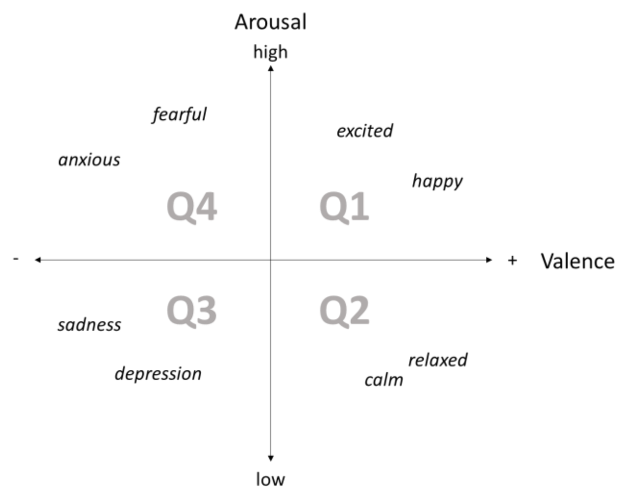

Figure 2.2 Depiction of the interaction of valence (horizonal axis) and arousal (vertical axis) makes up affective states (Q1, Q2, Q3, Q4). The words in italics indicate the possible locations of the reported affective states. Adapted from (Mendl et al. 2010) and (Crump et al. 2020).

2.2.1 Quantifying affective states using cognitive bias tests

Cognition can be described as the mechanism animals use to acquire, process, store and act on information from their environment (Roelofs et al. 2016). An animal's affective state can impact their cognitive abilities, which is called cognitive bias. Therefore, it is possible to infer an animal's affective state by assessing their cognitive bias. Three types of cognitive bias tests have been developed, including memory bias, judgment bias, and attention bias testing (Paul et al. 2005). A memory bias test relies on the fact that events connected to positive or negative emotions are remembered more frequently than neutral events (Kraemer et al. 2022). A judgement bias test investigates the propensity of an animal to show behavior that indicates anticipation for either a relatively positive- or negative- outcome in response to an ambiguous stimulus (Mendl et al. 2009).

For a judgement bias test, animals are trained to respond differently to two unidimensional stimuli, which leads to the valanced responses (Crump et al. 2018). Once trained, the animals are then introduced with an ambiguous intermediate stimulus and their response is used to infer valence states (Crump et al. 2018). If the animal responds to the stimulus in a positive way, this is interpreted as an optimistic judgement bias which is characteristic of a positive-valence state, whereas a negative response is interpreted as a pessimistic judgement bias indicative of a negative-valence state (Crump et al. 2018). Judgement bias tests do have a few shortcomings. The lengthy training periods can be time consuming for researchers, impractical for applied settings, and may lead to a selection bias (Crump et al. 2018).

Attention bias testing involves the simultaneous presentation of positive and negative stimuli and the observation of the animal's attention bias through vigilance behavior (Luo et al. 2019). In an ambiguous situation, animals with negative affective states will show negative attention bias, meaning they will show more bias towards a perceived negative stimulus than other stimuli present, thus they will be more anxious compared to animals in a more positive state.

The opposite can be observed in animals with a positive affective state. They will show more bias towards the positive or neutral stimuli present, and respond more calmly than animals in a negative state. Negative affective states, such as anxiety, can result in an attention bias towards a negative stimulus that appears threatening (Lee et al. 2018). A benefit of attention bias tests is that they require no training and are less time consuming compared to judgment bias tests (Crump et al. 2018).

Attention bias tests have been used in humans and a variety of animals and are summarized in Table 2.4. There is a general lack of application of the attention bias test in swine, with only 3 known sources that utilize it, all of which focus on weaning. While this event is stressful, there are several other stressful events related to management and using the attention bias test could be useful in investigating affective states of pigs in different life stages. Another known stressful even for pigs is mixing of new groups.

Table 2.4 Summary of studies using the attention bias test to evaluate the affective state of livestock. A longer latency to begin feeding coupled with increased vigilance behaviors indicates a more anxious animal and therefore a more negative affective state. (longer latency=more anxious; more time spent eating=less anxious)

Species	Stimuli	Affect manipulation	Measures of attention	N	Results	Reference
Laying hens	Mixed grain/ conspecific alarm call	N: Anxiogenic drug C: Saline	Latency to step, vocalize, and feed following first and second alarm call, number of steps and vocalizations, time spent feeding	50	• Latency to eat following first and second alarm call N > C • Latency to vocalize following second alarm call C > N; • Number of steps/ vocalizations N > C • Time spent feeding C > N • No effect on latency to step/vocalize following first alarm call or latency to step following second alarm call	(Campbell et al. 2019b)
	Mixed grain/ conspecific alarm call	N: Indoor ranging hens P: Outdoor ranging hens	Latency to step, vocalize, and feed following an alarm call playback (hens required to feed before the alarm call was played)	67	• Latency to step N > P • Latency to vocalize P > N • Greater number of N hens did not eat at all (never received an alarm call playback) compared to P hens ○ Of those N hens that initially feed, only 7% resumed feeding after the alarm call was played compared to 36% of P hens	(Campbell et al. 2019a)
Broilers	Mixed grain/ conspecific alarm call	1: High stocking density 2: Low stocking density 3: High complexity 4: Low complexity	Latency to begin feeding, resume feeding, first vocalization, and first step from the alarm call ended, occurrence of vigilance behaviors	Exp 1=60 Exp 2=144	• Exp 1: 1 = 2 = 3 = 4 • Exp 2: latency to eat 4 > 3; 3 > 4 more birds resumed feeding following playback of an alarm call	(Anderson et al. 2021)

Cattle	Hay/dog	N: Anxiogenic drug P: Anxiolytic drug C: Saline injection	Attention towards threat, vigilant behavior, number of zones crossed, urinations and defecations, and vocalizations, latency to feed, time spent feeding, ear posture, head shaking, tail swishing	36	• Attention towards threat/vigilant behavior/backwards ear posture N > C & P • Latency to feed N > C & P • Time spent feeding C & P > N • Head shaking/tail swishing/defecating/zones crossed N > C & P	(Lee et al. 2018)
Sheep	Familiar feed bucket	N: Low-feeding P: High-feeding	Latency to first detection, to approach, time spent interacting with stimuli, interaction with environment, vocalizations	41	• Latency to approach N > P • Interacting with bucket and environment N > P • No effect on latency to first detection, latency to approach, or vocalizations	(Verbeek et al. 2014)
	Feed/dog	N: Anxiogenic drug P: Anxiolytic drug C: Saline	Freezing behavior, attention towards the threat, vigilance behavior, zones crossed, latency to feed, time spent feeding	60	• Attention towards threat/vigilant behavior N > C > P • No effect on zones crossed or vocalizations • No N sheep fed, with no difference between P & C	(Lee et al. 2016)
	Hay/dog	N: Anxiogenic drug P: Anxiolytic drug C: Saline	Latency to eat, duration of vigilance/attention to threat, time spent eating, number of zones crossed	60	• Duration of vigilance N > P & C • Attention to threat N and C > P • No effect on zones crossed or vocalizations	(Monk et al. 2018)

	Familiar food bucket/ dog	S: Anxiogenic drug C: Saline injection	Duration of attention towards threat, vigilance, latency to eat, number of zones crossed, time immobile	27	• No effect on duration of attention towards threat, vigilance, latency to eat, number of zones crossed, time immobile	(Monk et al. 2019)
	Food/ unfamiliar dog	S: rest disruption and individual housing C: group housing with low stress handling	Number of zones crossed and vocalizations, latency to eat, amount of food eaten, duration of attention to threat, looking at the food and vigilance behaviors	60	• Vigilance behavior C > S • Latency to eat S > C • Food consumed S > C • No effect on duration of attention to threat, looking at the food	(Verbeek et al. 2019)
	Recordings of sheep bleating/dog barking	N: Negative mood (i.e. various unpredictable/ uncontrollable events) P: Positive mood (i.e. conditions better than minimal commercial standards)	Head and ear positions and movements	32	• N sheep shifted their attention towards dog barking when it was at a lower volume than the sheep bleating and shifted their attention towards sheep bleating when the recordings were the same volume • P sheep shifted attention towards of barking when both recordings were of low intensity	(Raoult & Gygax 2019)
	Photograph of a sheep/dog	N: Anxiety-inducing drug P1: Calm-inducing drug P2: Happy-inducing drug C: Saline	Attention towards both stimuli, vigilant behavior, number of sniffs of photo/environment, zones crossed, vocalizations	80	• Vigilant behavior N > C, P1, & P2 • Number of sniffs of photo and environment N < C, P1, & P2 • Zones crossed P1 & P2 > N • Urinations N > C, P1, & P2 • No effect on attention towards dog or vocalizations	(Monk et al. 2020)

	Feed pan/ flashing light & moving door	1: Enriched 2: Barren 3: Enriched to barren 4: Barren to enriched	Attention towards threat, vigilant behavior, latency to eat, time spent eating, vocalizations, urinations and defecations	128	• No effect on attention towards threat, vigilant behavior, or time spent eating • Latency to eat 1 & 4 < 2 & 3 • Vocalizations 1 & 4 > 2 & 3 • No effect on defecation or urination	(Luo et al. 2019)
	Conspecific area + toys/ aggressive dog bark	P: Lactobacillus supplementation C: Placebo	Zones crossed, attention to threat, vigilant behavior, exploratory behavior, locomotion, interacting with toys	64	• Vigilant behavior C > P • Attention to threat P > C • No effect on zones crossed, exploratory behaviors, locomotion, or interactions with toys	(Verbeek et al. 2021)
Pigs	Feed/ flashing light + siren	1: No black soldier fly larvae pre- or post-weaning 2: Black soldier fly larvae pre-weaning only 3: Black soldier fly larvae post-weaning only 4: Black soldier fly larvae pre- and post-weaning	Attention to threat, vigilant behavior, locomotion, exploratory behavior, interacting with environment, interacting with feed, vocalizations, urinations and defecations	96	• Attention to threat 2 > 1 • No treatment effect on vigilant behavior, locomotion, exploratory behavior, interacting with environment, interacting with feed, vocalizations, urinations and defecations	(Ipema et al. 2022)

2.3 Identifying pain in piglets

Surgical castration without analgesic is a painful experience for piglets. Pain assessment and recognition could be a valuable tool to sensitize producers and farm employees to piglet pain experience, and can be possibly used as a tool for pain management procedures. Pain recognition may lead to more willingness to apply analgesics or use of alternative castration methods. Several physiological indicators of pain in piglets include increased adrenocorticotropin hormone, cortisol, and lactate levels as well as higher body temperatures (Table 2.2) (Lonardi et al. 2015, Gottardo et al. 2016). However, assessing these indicators of pain in piglets is both time-consuming, invasive, and expensive. Behavioral indicators provide a non-invasive, easy-to-use pain assessment tool for swine producers. Validated behavioral methods for piglet pain assessment include the Piglet Grimace Scale, tail motion and posture, vocalizations, and piglet activity. Although most of these methods are not regularly used within the industry, they could be useful as tools for making pain management decisions associated with not only castration but all processing procedures that occur for piglets.

2.3.1 The Piglet Grimace Scale

One way to determine a piglet's pain experience is by quantifying their facial expressions. Species-specific grimace scales are pain assessment tools that could be used as a decision tool for analgesia application and as an indicator of animal welfare (Miller & Leach 2015). A Piglet Grimace Scale was developed based on 10 facial action units (FAUs) – temporal tension, forehead profile, orbital tightening, cheek tension, tension above the eyes, lower jaw profile, upper lip contraction, snout plate changes, snout angle and nostril dilatation – each ranked on a 0-2 scale (0=not present, 1=moderately present, 2=obviously present) (Di Giminiani et al. 2016). However, when this scale was applied, only “orbital tightening” was shown to significantly change due to tail docking (Di Giminiani et al. 2016). In 2017, another, more refined Piglet Grimace Scale (PGS) was developed and validated as a scoring system to recognize pain in piglets after surgical castration (Figure 2.3) (Viscardi et al. 2017, Viscardi & Turner 2018). The PGS is an ordinal scale focusing on three FAUs – ear positioning, cheek tightening, and orbital tightening – with higher scores representing more severe pain expression. The ear position is based on a 0-3 scale, cheek tightening/nose bulge on a 0-2 scale, and orbital tightening on a 0-1 scale. Castrated piglets showed more pain behaviors and had higher PGS scores (more facial grimacing) compared to uncastrated piglets (Viscardi & Turner 2018).

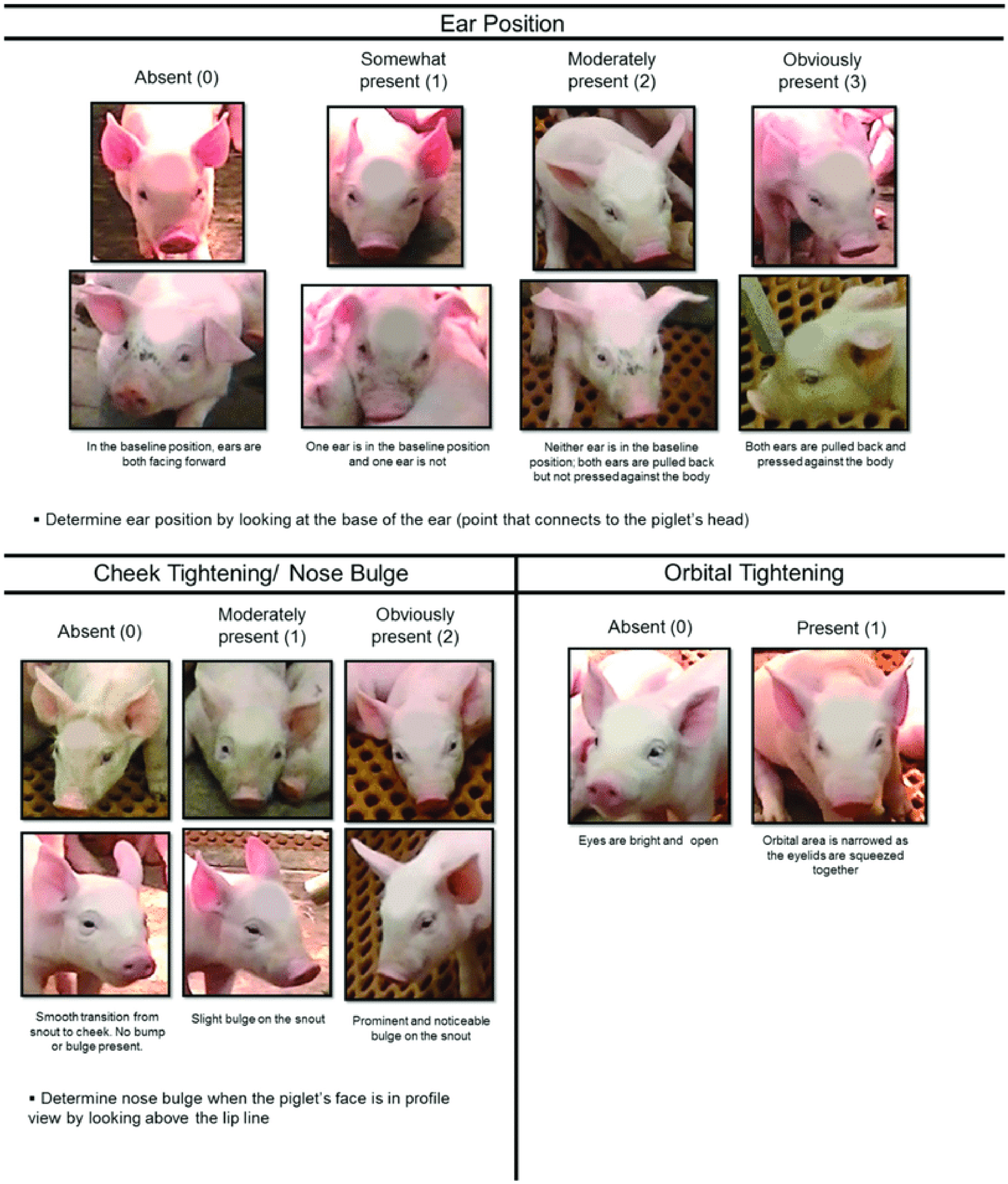

Figure 2.3 The Piglet Grimace Scale (PGS) is based on the scoring of the ear positioning, cheek tightening, and orbital tightening (Viscardi & Turner 2018).

2.3.2 Tail posture and motion

Regardless of species, an animal's tail posture and motion play a key role in communication of emotion and affective state. Specifically, an erect tail in deer is an example of tail posture acting as a warning sign (Stankowich 2008) and tail flagging in squirrels is an example of tail motion (Barbour & Clark 2012). Tails have also been used as a threat display in snakes (Brown et al. 2020) and used is mating rituals in birds (Scholes et al. 2017). Tail motion and posture are at least partially related to emotional state (Camerlink & Ursinus 2020). Thus, posture and motions can be placed in the model of affect based on their interaction between arousal and valence (Figure 2.4). Because of this, the tail could be useful in welfare assessments, for instance in the case of tail biting (Camerlink & Ursinus 2020). The different tail postures and motions are described in Table 2.5.

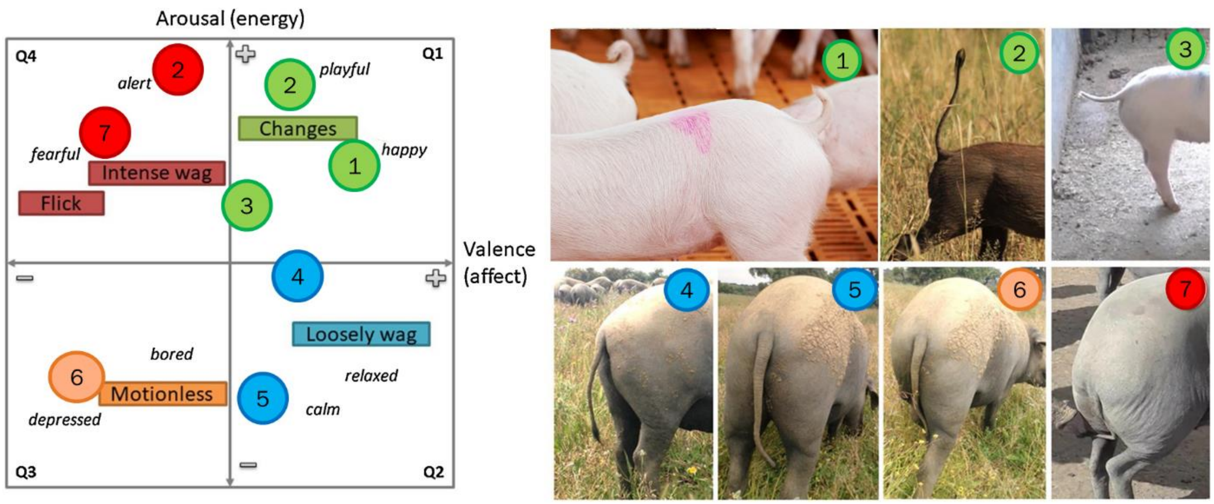

Figure 2.4 An approximation of where tail motions (rectangles) and tail postures (circles) can be placed in the model of affect (Mendl et al. 2010, Crump et al. 2020). The numbers relate to the pictures of different tail postures and affective states are placed where they are commonly described in literature. Adapted from (Camerlink & Ursinus 2020).

Table 2.5 Ethogram for recording tail postures and motions along with a reference to the example pictures given in Figure 2.4. Adapted from (Camerlink & Ursinus 2020).

Tail	Description	Image
	Posture	
Curled	The base of the tail is held horizontally with the remainder of the tail rotating downward	1
Erect/Upright	The tail is held straight upright perpendicular to the spine at about 90°	2
Horizontal	The tail is sticking straight out from the body, aligned with the spine	3
Active Hanging	The full tail is pointing loosely downward whereby there is at least a 30° angle between the base of the tail and the hind parts, resulting clearly in space between the tail and the body	4
Passive Hanging	The full tail is pointing downward little or with no space between the tail and body. The tip of the tail is not between the legs.	5
Tucked	The full tail is kept vertically down and is kept close to the body whereby the tip of the tail is held in between the legs.	6
Jamming	The tail is suddenly contracted towards the body whereby the tip of the tail is held in between the legs. The muscles around the base of the tail are tightened.	7
	Motion	
Motionless	The tail is not moving within its posture	
Loosely Wag	The tail moves gently from side to side, with 1−3 movements per second	
Intensely Wag	The tail moves rapidly from side to side, with 4−7 movements per second. Including sideways tail shaking other than flicking.	
Flicking	The tail moves rapidly in sudden up and down or sideways motion in a single 'tail flick'.	
Circling	The base of the tail is rapidly rotating causing the remainder of the tail to move in a circular fashion.	

2.3.3 Vocalizations

Vocalizations produced by different species show acoustic characteristics that vary depending on the emotional content or their physiological states (Manteuffel et al. 2004, Briefer & le Comber 2012). Emotions have an impact on vocalizations and are therefore becoming increasingly considered as a useful, non-invasive tool to assess animal affect (Briefer & le Comber 2012). Researchers developed a system to automatically recognize acoustic indicators of stress states in pigs (Schön et al. 2004). This system detects differences in the frequency of vocalizations (i.e., high- vs low frequency) and then classifies them as stress or no-stress calls. Studies on vocalizations as an indicator of emotions have restricted to specific call types by the animal at a certain age, in a specific environment, and experiencing well-defined – albeit limited – scenarios (Briefer & le Comber 2012). Pigs have a highly developed vocal communication system and the acoustic features of the different vocalizations vary according to the context of production (Tallet et al. 2013). Part of the acoustic variations may reflect the interaction between valence and arousal (Briefer et al. 2022). Previous research has shown that pig vocalizations can be classified into high- and low-frequency call; each having two or three less distinct subcategories (Tallet et al. 2013). High-frequency calls (screams, squeals) are common in negative situations while low-frequency calls (grunts) are common during neutral and positive situations (Tallet et al. 2013). Several studies have found differences in piglets' calls while undergoing a painful procedure as opposed to handling alone, including surgical castration and are summarized in Table 2.6. These variations within call-type could potentially be used to interpret the associated emotion or affective state, as well as the context of emission (Briefer et al. 2022). Meaning if a specific call-type can be shown to be consistently emitted during castration, then it could provide insight into a piglet's affective state and may even be useful for recognizing when analgesics are needed.

Table 2.6 Summary of studies investigating call types of piglets during painful procedures. An increase in vocalizations indicates distress and a more negative emotion.

Treatments	**N**	**Vocalization measurements**	**Results**	**Reference**
TE: Teeth clipping only TA: Tail docking only E: Ear notching only TTE: Teeth, tail, and ears C: Handling	279	Frequency of vocalizations from 0-30s, 30-60s, and 60-90s post procedure	• TA > TE, E, TTE > C vocalization frequency 0-30s • TA, TTE > E, TE, C vocalization frequency 30-60s • No difference 60-90s	(Noonan et al. 1994)
C: Castration M: Castrated with analgesia L: Castrated with local anesthesia LM: Castrated with both analgesia and local anesthesia	141	Vocalization intensity during castration	• C, M > L, LM call intensity • No difference between L and LM or C and M	(Hansson et al. 2011)
N: Surgical castration after receiving N_2O C: Surgically castrated with air	24	Total vocalization length during the induction phase before castration and total vocalization length during the castration procedure	• C > N vocalizations before and during castration	(Rault & Lay 2011)
C: Castrated CA: Castrated with short-acting anesthetic CL: Castrated with long-acting anesthetic S: Sham-handled	40	Percentage of stress vocalizations	• C > CA, CL, S % stress vocalizations	(Sutherland et al. 2010)

Chapter 3: Investigating the short- and long-term impacts of pain from surgical castration on affective states in piglets

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3.1 Abstract

Surgical castration of male piglets is a routine procedure performed to improve meat quality and reduce aggression as piglets age. Pain due to castration can last for up to 4 days, negatively impacting animal welfare. Thus, feasible approaches for pain alleviation are needed. The objective of this study was to assess the impact of surgical castration and practical pain alleviation methods during and after castration on piglets' short and long-term affective states and activity. Piglets were surgically castrated (n=22; CAS), castrated with analgesics (n=21; CAS-A), or sham-handled (n=22; SHAM) at 3 days of age. Data on piglets' activity (using accelerometers), tails (affective states), and grimaces (pain expression) were collected for 1, 6, and 24 hours after castration treatments while piglets were in their home pens. An attention bias test was performed in week 1 (n=31; WK1) and 12 (n=33; WK12) to assess anxiety (an affective state), with feed (positive stimulus) and loud bangs and flashing lights (negative stimuli) presented simultaneously. Latency to eat, behavioral responses, and activity (using accelerometers) were recorded during the 3-min test. Activity measures were assessed using mixed models with treatment as fixed factor and piglet identification as random factor. Piglet grimace scores, tail postures and motion were analyzed as total scores using ordinal logistic models with treatment and time since castration as fixed factors.

Frequency of attention bias test responses were analyzed using ordinal logistic models with treatment and test week as fixed factors. Duration of attention bias test responses were analyzed with mixed models by week with treatment as a fixed factor, and farrowing batch and piglet identification as random factors. Number of piglets eating was assessed with an ordinal logistic model with treatment and test week, and their interaction as fixed factors. Responses were analyzed with mixed models by week (either WK1 or WK12) with treatment as a fixed factor and piglet ID and farrowing round as random factors. Castration treatment and sampling timepoint impacted forward and vertical activity in the home pen. Latency to eat did not differ between treatments in WK1. CAS piglets spent less time eating compared to the SHAM group in WK1 ($P=0.019$). CAS-A piglets tended to spend more time being vigilant compared to SHAM piglets in WK1 ($P=0.075$). Piglets tended to show more forward activity in CAS (2.37 ± 0.29 m/s^2) compared to CAS-A (1.43 ± 0.27 m/s^2; $P=0.067$) piglets in WK1. Treatments did not impact responses during the attention bias test in WK12. The piglets' behavior (eating) and activity suggest a negative impact of CAS compared to SHAM and CAS compared to CAS-A on affective states. Vigilance responses showed a negative impact of CAS-A compared to SHAM, but did not show an impact of CAS on vigilance. Any of these differences observed in WK1 were no longer detected in WK12, suggesting that any potential impact of surgical castration on affective states at 3 days of age is no longer detectable at 12 weeks of age. As this is the first study assessing the relationship between piglet anxiety and painful procedures, more research is needed to better understand the impact of these procedures on the affective state.

Keywords: animal welfare, attention bias, piglet castration, pain alleviation

3.2 Introduction

All male piglets undergo invasive husbandry procedures within the United States (U.S.) swine industry, including surgical castration. The primary reason for castration is to prevent the development of boar taint in meat, which is a foul odor when the meat is prepared caused by the post-pubertal deposition of skatole and androsterone in body fat (Keenan 2016). In the U.S., carcasses that give off a pronounced boar taint are condemned for human consumption (9 CFR 311.20 - Sexual odor of swine. 2012). If the odor is less pronounced, the carcass may be used for comminuted cooked meat products or for rendering. Producers in the U.S. will surgically castrate the boars to prevent boar taint development and avoid potential economic consequences associated with carcass rejection or downgrading.

Surgical castration is typically performed by trained farm employees. The American Veterinary Medical Association (AVMA) recommends that after 14 days of age, boars should be castrated using analgesia, anesthesia, or both (AVMA 2013). However, piglets in the U.S. swine industry are castrated within the first week of life so no analgesia or anesthesia is used even though the procedure is painful (Rault et al. 2011). Pain is a combination of physical and emotional reactions to a stimulus, making it an affective experience (Steagall et al. 2021). Therefore, the pain experienced during surgical castration could possibly impact piglets' short and long-term affective states.

Affective state can be defined as a long-lasting mood state resulting from an accumulation of subjective experiences and emotions and can range between negative and positive in valence (Mellor 2014). An animal's affective state can impact their cognitive abilities, which is called cognitive bias. Therefore, it is possible to infer an animal's affective state by assessing their cognitive bias (Roelofs et al. 2016). Three types of cognitive bias tests have been developed, including memory bias, judgment bias, and attention bias testing (Paul et al. 2005). Attention bias testing involves the simultaneous presentation of positive and negative stimuli and the observation of the animal's attention bias through vigilance behavior (Luo et al. 2019). In an ambiguous situation, animals in negative affective states will show more bias towards a perceived negative stimulus than other stimuli present (Lee et al. 2018) and thus will be more anxious compared to animals in a more positive affective state. The opposite can be observed in animals in a positive affective state, where they will show less bias towards the negative stimulus and respond more calmly than animals in a negative state. Attention bias tests have been used in humans (Tanda et al. 2022) and animals, including sheep (Lee et al. 2016, Rodger et al. 2018, Monk et al. 2020), cattle (Crump et al. 2018), poultry (Campbell et al. 2019b, 2022, Anderson et al. 2021), and pigs (Luo et al. 2019, Verbeek et al. 2021, Ipema et al. 2022). In one study, pigs biased their attention to a threat and were more vigilant than pigs that were tested in the same arena without a threat (Luo et al. 2019). This suggests that the threat induced an anxiety response, rather than any other aspects related to the test environment. The same researchers investigated the effects of early and later in life housing conditions on attention bias and found that pigs housed in enriched pens later in life were more anxious compared to those housed in barren pens (Luo et al. 2019). Another study used an attention bias task and showed that *Lactobacillus* supplementation reduced anxiety-associated behaviors, improving affective states in piglets compared to a control diet (Verbeek et al. 2021). Although not fully validated, these studies indicate that attention bias testing can be used as a tool to determine pigs' affective states.

Surgical castration causes behavioral and physiological responses indicative of acute and chronic pain (Prunier et al. 2005, Sutherland et al. 2010, Lonardi et al. 2015, Gottardo et al. 2016). However, the impact of surgical castration on short-term and long-term affective states is unclear. Therefore, the objective was to evaluate the short- and long-term impact of pain from surgical castration on pigs' affective state. We hypothesized that surgically-castrated pigs would bias their attention towards the threat compared to sham-handled pigs or pigs castrated with analgesics at 1 week of age. Furthermore, we theorized that any long-term impacts of surgical castration pain would be reflected in the behavioral responses during an attention bias test at 12 weeks of age.

3.3 Methods

The use of the boars in this study was approved by Virginia Tech Institutional Animal Care and Use Committee, protocol #20-206.

3.3.1 Animals and Housing

Sixty-five day-old boars (York × Landrace) originated from 11 litters born at the Virginia Tech Swine Center. Litters were farrowed in batches, with two litters in March 2021, three litters in April 2021, and six litters in August 2021. The sows were moved into conventional farrowing crates 3-6 days before the first sow in the batch was due to farrow.

On day 1 of age, boars were selected based on the number of boars in a litter (≥6) after which they were weighed and ear tagged. If there were more than six boars in a litter, the heaviest six males were included in the study. On day 1 and at weaning, males were administered 1 ml of CIRCUMVENT® PCV-M G2 vaccine (Merck Animal Health, Omaha, Nebraska, USA) through intermuscular injection. Males were administered 2 ml of Iron Hydrogenated Dextran (VetOne, Boise, Idaho, USA) through intermuscular injection on day 1 and were vaccinated with 2 ml of Rhini Shield TX4 (Elanco, Fort Dodge, Iowa USA) at weaning.

Sows and litters were housed in conventional farrowing crates (2.4 m × 1.5 m) until boars were weaned at (mean ± SD) 19.8 ± 1.4 days of age. Sows were fed 3 kg of a standard commercial lactation diet once per day prior to farrowing and twice per day after farrowing. While in the farrowing crate, piglets had continuous access to a water nipple and a heating mat until weaning. Piglets also had access to a commercial creep feed starting 7 days prior to weaning.

After weaning, males from the same farrowing batch were housed together in wire-floored nursery pens (2.4 m × 1.2 m) containing a nipple drinker and a feeder, at a stocking density of 3-4 piglets/m^2. Pigs had *ad libitum* access to a commercial nursery diet. After (min-max) 25-31 days in the nursery, males were moved to grower pens. Males from the same farrowing batch were housed together in partially-slatted concrete grower pens (2.6 m × 3.0 m) with two nipple drinkers and a feeder, at a stocking density of 1-2 pigs/m^2. Pigs had *ad libitum* access to a commercial grower diet.

In the third farrowing batch, tail biting was observed at 44-46 days of age. All piglets with tail lesions were treated with 0.5 ml of ceftiofur crystalline-free acid (Excede®, Zoetis, Kalamazoo, MI, USA). Thereafter, all nursery pens received two Kong toys (Kong Company, Golden, Colorado, USA), and a herding ball (Jolly Pets, Streetsboro, OH, USA). Thereafter, all grower pens were equipped with a suspended plastic teething ring (QC Supply LLC, Schuyler, Nebraska, USA) along with the two Kong toys and ball.

3.3.2 Treatments

This study included three treatment groups, with males undergoing surgical castration (negative control; CAS), males receiving analgesia prior to and after surgical castration (pain relief; CAS-A), and males sham-handled and not castrated (positive control; SHAM).

Piglets were randomly allocated to one of three treatment groups within a litter using their identification number on day 1. On day 3 of age, the six boars from a single litter were removed from the farrowing crate and placed in a holding cart. A single trained technician performed all castration treatments and handling. Piglets were arbitrarily chosen from the holding cart prior to treatment, castrated or handled, and returned to cart. Once all boars from a single litter were treated, they were returned to the farrowing crate.

In the CAS treatment, individual boars (n=22) were restrained between the technician's legs. Piglets were surgically castrated by making two vertical incisions on the scrotum and cutting the spermatid cord with a scalpel blade. These piglets were surgically castrated without anesthesia or analgesia, as is common in the U.S. swine industry (Rault et al. 2011). The CAS procedure took (mean ± SD) 31 ± 4 sec. Piglets in the CAS-A treatment (n=21) were surgically castrated as in the CAS treatment, with additional analgesics. A topical lidocaine spray (Sanifo Company, Chattanooga, Tennessee, US) was applied, and the technician waited 5 sec prior to the incisions on the scrotum. The CAS-A procedure took (mean ± SD) 32 ± 4 sec. Following castration, the

CAS-A piglets were administered flunixin (VetOne, Boise, Idaho, USA) at 2.2 mg/kg once a day for three days. Piglets in the SHAM treatment (n=22) were handled similarly as in the CAS treatment; piglets were restrained between the technician's legs for 30 sec. The SHAM procedure took (mean ± SD) 30 ± 0 sec.

3.3.3 Measurements

The project timeline is provided in Figure 3.1. In short, data were collected on body weight, activity, pain expression, and affective state including anxiety.

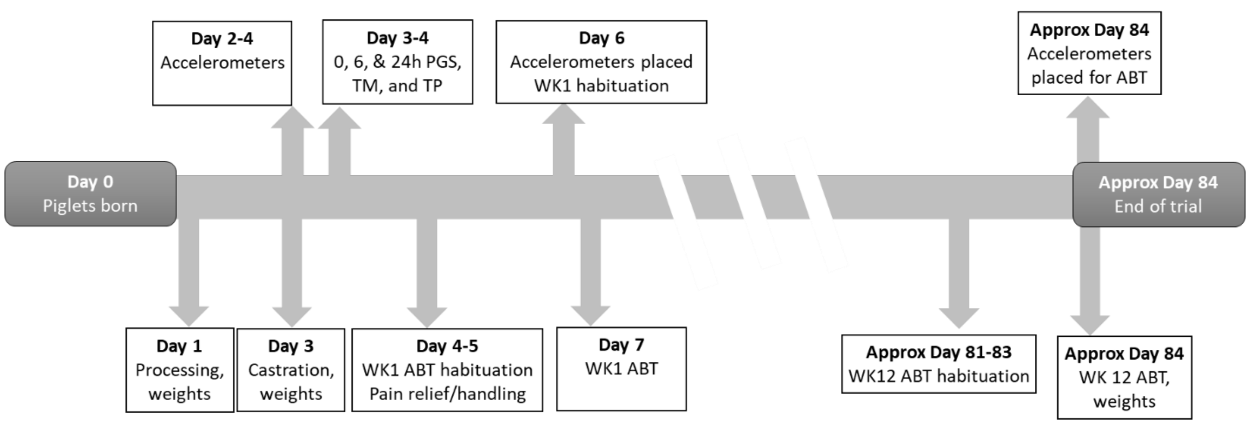

Figure 3.1. Timeline for the study measurements. (PGS = Piglet Grimace Scale, TM = tail motion, TP = tail posture, WK1 = Week 1; WK12 = Week 12; ABT = attention bias test)

Body Weight

Individual body weights were recorded at day 1, day 3, weaning (day 20 ± 1 of age), and the last day of the trial (day 84 ± 3 of age) for all piglets.

Activity

On day 2 of age, accelerometers (HOBO Pendant® G Data Logger, Onset Computer Corporation, Bourne, MA, USA) were placed on the piglets' back and secured with vet wrap (Andover Healthcare, Salisbury, MA, USA) around their torso and front legs. The accelerometers were programmed to start recording the following day (day 3 of age) to allow for habituation to the vet wrap and accelerometer prior to data collection. Activity data while in the farrowing crate (home pen) were collected on the X (forward activity) and Z (vertical activity) axes in m/s^2 at intervals of 15 sec for 1 hour prior to castration treatments and 1, 6, and 24 hours after castration treatments, resulting in 4 hours of activity data and 960 data points per piglet. In addition, accelerometers were used to record activity during the 3-minute attention bias test at week 1 and week 12 of age. On day 6 of age, the vet wrap and accelerometers were removed. For the piglets tested at week 12 of age, vet wrap and accelerometers were reattached prior to the attention bias test.

Pain Expression and Affective States

Within 1 hour after treatment, and again after 6 and 24 hours, the grimace (indicative of pain expression (Viscardi & Turner 2018), and tail posture and tail motion (indicative of affective state (Camerlink & Ursinus 2020) were video recorded (EOS Rebel T7 DSLR Camera, Canon, Tokyo, Japan) while piglets remained in the farrowing crate with the sow. If any of the treated piglets were sleeping, the sow was gently stimulated to stand or offered some feed in order to arouse the piglets. Data were not collected from piglets that were nursing or continued to sleep at that specific time point. From the video recordings, piglets were identified and time points were noted in which grimace or tail were clearly visibly for scoring. All observers were blinded for castration treatment. A total of 165 grimaces and 176 tail postures and motions were scored (Table 3.1).

Table 3.1. Sample size for piglet face captures from video for the piglet grimace scale scoring and for piglet tail posture and motion captures for affective state assessment 0, 6, and 24 hours after treatment in surgically castrated pigs (CAS), castrated pigs that received analgesia (CAS-A), and sham-handled pigs (SHAM).

Timepoint	Piglet grimace capture (n)				Tail posture and motion capture (n)			
	Treatment			Total	Treatment			Total
	CAS	CAS-A	SHAM		CAS	CAS-A	SHAM	
0 hours	19	17	18	54	19	20	20	59
6 hours	19	18	18	55	21	18	21	60
24 hours	19	18	19	56	22	18	17	57
Total	57	53	55	165	62	56	58	176

Piglet pain expression was scored by two observers using the piglet grimace scale (PGS; Viscardi & Turner 2018), which consists of a 0-6 categorical scoring system with scores of 0–1 representing a piglet experiencing "no-to-low pain" and scores of 4–6 representing "moderate-to-high pain" (Viscardi et al. 2017, Viscardi & Turner 2018). Inter-rater agreement was tested for 14 piglet grimaces that were not included in this study and the agreement was strong among observers (Cronbach's $\alpha = 0.7531$).

Affective states were assessed from tail postures and motions using a modified method described by Camerlink & Ursinus (2020). Piglets' tails were kept intact, thus were not docked. Seven tail postures (rather than eight in Camerlink & Ursinus 2020) and five types of tail motion (Camerlink & Ursinus 2020) were recorded and represented varying combinations of arousal and emotional valence (Table 3.2; Q1: high arousal, positive valence; Q2: low arousal, positive valence; Q3: low arousal, negative valence; Q4: high arousal, negative valence; Camerlink & Ursinus 2020). Two trained observers showed an excellent inter-rater agreement (Cronbach's $\alpha = 0.9379$) based on scores for 25 piglet tail postures not included in this study and both observers scored all pigs. Two other trained observers showed an excellent inter-rater agreement (Cronbach's α = 0.9028) based on scores for 20 piglet tail motions not included in this study, and both observers scored all pigs.

Table 3.2. Tail postures and motion categories as modified from Camerlink & Ursinus (2020) and their association with emotional valence, arousal and the quantile in the circumplex model of affect. (+ = positive; - = negative)

Tail characteristic	Description	Valence	Arousal	Quantile in the circumplex model of affect[1]
	Posture			
Curled	The base of the tail is held horizontally with the remainder of the tail rotating downward	+	+	Q1
Horizontal	The tail is sticking straight out from the body, aligned with the spine	+	+	Q1
Erect	The tail is held straight upright perpendicular to the spine at about 90°	+/-	+	Q1/Q4
Active hanging	The full tail is pointing loosely downward whereby there is at least a 30° angle between the base of the tail and the hind parts, resulting clearly in space between the tail and the body	+	-	Q2
Passive hanging	The full tail is pointing downward little or with no space between the tail and body. The tip of the tail is not between the legs.	+	-	Q2
Tucked	The full tail is kept vertically down and is kept close to the body whereby the tip of the tail is held in between the legs.	-	-	Q3
Jamming	The tail is suddenly contracted towards the body whereby the tip of the tail is held in between the legs. The muscles around the base of the tail are tightened.	-	+	Q4
	Motion			
Motionless	The tail is not moving within its posture	-	-	Q3
Loosely Wag	The tail moves gently from side to side, with 1−3 movements per second	+	-	Q2
Intensely Wag	The tail moves rapidly from side to side, with 4−7 movements per second. Including sideways tail shaking other than flicking.	-	+	Q4
Flicking	The tail moves rapidly in sudden up and down or sideways motion in a single 'tail flick'.	-	+	Q4
Circling	The base of the tail is rapidly rotating causing the remainder of the tail to move in a circular fashion.	-	+	Q4

[1] Q1: high arousal, positive valence; Q2: low arousal, positive valence; Q3: low arousal, negative valence; Q4: high arousal, negative valence (Camerlink & Ursinus 2020)

Anxiety

At week 1 and week 12 of age, the piglets' (n=61) attention bias was determined as an indicator for anxiety in a test modified from (Luo et al. 2019). Castration treatments were balanced across the 1-week-old test group (WK1; n=32) and the 12-week-old test group (WK12; n=29).

A 3 m × 3 m × 1 m (length × width × height) arena with a 1 m × 0.6 m start box was constructed from plywood, placed on a concrete floor in a separate building from where the farrowing crates or pens were located (Figure 3.2).

First, pigs were allowed to habituate to the arena during three sessions, three days prior to the test (days 4 - 6 of age for WK1 piglets and days 77 - 89 of age for WK12 piglets). At 4 and 5 days of age, all three WK1 littermates were placed in the arena together for 3 min. At 6 days of age, WK1 piglets were individually placed in the arena for 3 min. Similarly, WK12 pigs from the same grower pen were placed in the arena for 3 min in the first two habituation sessions, and individually in the last habituation session (also outlined in Figure 3.1). During habituation, no threat or feed were presented.

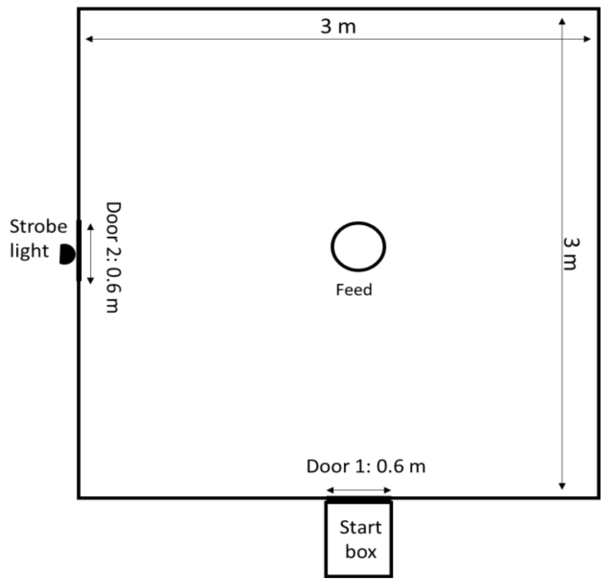

Figure 2. Top-view illustration of the attention bias test arena. Door 1 separated the arena and the start box in which the pig was placed at the beginning of the test. Prior to the test, door 1 was opened and the pig was allowed to move into the arena. Door 2 was a guillotine door which served as the unknown threat (opened and closed repeatedly) in combination with the strobe light. Feed was placed in a familiar feed bowl in the center of the arena.

A day after the third habituation session, pigs' attention bias was individually tested for 3 min. Testing order was randomly predetermined based on piglet identification number within a farrowing batch. At day of testing, the test arena contained a familiar metal feeding pan located in the center of the arena with an electrolyte solution (Gatorade, The Gatorade Company, Chicago, IL, USA) in week 1 and a mixture of raisins, chocolate chips and apple slices in week 12. Gatorade is a non-carbonated sports drink with water and sugar as the main ingredients and is known to be voluntarily ingested by piglets at pre-weaning ages (Lin 2020). The threat consisted of a guillotine plywood door that was repeatedly opened and closed loudly (aversive sound repeated approximately 9 times) and a strobe light shining across the arena (aversive light) for 10 sec at floor level. This threat produced flashing lights, squeaking noises, and loud bangs.

During the attention bias test, the piglet was placed in the start box, and after approximately 10 sec the door was opened and the piglet was allowed to exit the start box and enter the test arena. Once the start box door was closed, the test started. The threat was presented after the piglet looked at the guillotine door (Figure 2) or after 10 sec passed. During and after the threat, behavior was recorded live and from video recordings. Live observations included the latency to eat (sec) and whether the piglet ate (yes/no), frequency of urination and defecation, frequency of escape behavior, frequency of vigilance, frequency of attention to threat, and frequency of vocalizations including barks, grunts, and high-pitched vocalizations as described by (Luo et al. 2019) (Table 3). One observer recorded frequency of attention to threat, frequency of urination and defecation, and latency to eat (sec). A second observer recorded frequency of escape behavior and frequency of vigilance. A third observer recorded the frequency of all vocalizations. All observers were blinded to treatments.

Video-based behavioral observations were performed to determine behavioral durations (sec) and frequencies (% of total observations; Table 3.3). Two trained observers recorded these behaviors and were blinded to the treatments. The observers showed an excellent inter-rater agreement (Cronbach's $\alpha = 0.9981$).

Table 3.3. Ethogram used to score the behavior of the piglets during the attention bias test.

Behaviors	**Definition**
Attention to threat	Head oriented towards the threat (door 2; Figure 1)
Vigilance	Standing motionless with head at shoulder, higher, or lower[1]
Eating	In contact with the feed man or feed with nose or mouth. With or without chewing.
Exploring	Sniffing, nosing, rooting or licking any component of the environment, excluding the feed. This can be performed with or without locomotion.

Locomotion	Walking without performing any other behavior. Some or all four legs move in a similar direction, this includes turning[1]
Standing	Standing with four paws on the floor without performing any other described behavior[1]
Laying down	Piglet's belly is in contact with the ground
Escape	Piglet is trying to escape the arena, usually by climbing or jumping at the wall[1]
Urinate/defecate[2]	Discharge of feces or urine
Other	Piglet shows behavior other than described
Out of sight	Piglet cannot be observed
High-pitched vocalizations[2]	Screams, squeals, or grunt-squeals[1]
Grunt[2]	Short guttural sound
Bark[2]	A low tone that sounds like a "wuff"[1]

[1] Behavioral definition from Luo et al. 2019.

[2] Recorded as frequency of event.

3.3.4 Statistical Analysis

Statistical analyses were conducted using JMP Pro 16 (SAS Institute Inc., Cary, NC, USA). Pigs were considered the experimental units. Data residuals were assessed for normality using normal quantile plots. Residuals for the duration of exploring and frequency of attention to the threat showed normal distribution. Residuals for body weights, all other behavioral durations and frequencies, all activity data, number of piglets that ate, tail posture and motion, and all vocalizations did not show a normal distribution. Even though residuals for those data were not normally distributed, the use of mixed-effects models is appropriate as these are robust to quite severe violations of model assumptions such as the residuals' distribution (Schielzeth et al. 2020), and allow for more complex models including random effects. Statistical models are detailed in Table 3.4.

Table 3.4. Statistical models applied for each response variable.

Response variable	Model	Fixed	Random
Body weights	Mixed	Treatment, age, interaction	Piglet identification
Activity during the attention bias test	Mixed	Treatment (by week)	Piglet identification
Attention bias behavior (duration/latency)	Mixed	Treatment (by week)	Piglet identification, farrowing batch
Attention bias behavior (frequency/number of piglets)	Ordinal logistic	Treatment, test week, interaction	
Grimace scale scores, tail postures, tail motions	Ordinal logistic	treatment, time since castration, interaction	

Interaction effects at $P<0.10$ were removed from the models. Post-hoc comparisons were performed with Tukey HSD corrections for effects with $P<0.10$. Escape, laying down, tail circling, WK1 high-pitched vocalizations, and barks were rarely observed and therefore not analyzed (frequency of 7 or lower). Data are presented as LSmeans ± SEM unless otherwise noted.

3.4 Results

3.5.1 Body Weights

Piglet body weights on day 1, day 3, weaning, and day 84 did not differ across treatment groups ($P=0.342$; Table 3.5).

Table 3.5. Mean individual body weights (raw mean ± SD; in kg) for surgically castrated pigs (CAS; n=22), castrated pigs that received analgesia (CAS-A; n=21), and sham-handled pigs (SHAM; n=22) at day 1 of age, day 3 of age, at weaning, and at day 84 of age.

Treatment	Day 1	Day 3	Weaning	Day 84
CAS	1.61±0.24	1.99±0.36	5.66±1.68	27.60±5.79
CAS-A	1.65±0.22	2.02±0.31	6.08±1.74	26.92±7.81
SHAM	1.53±0.23	1.86±0.36	5.57±1.63	24.40±7.75

3.5.2 Home Pen Data

Activity Measurements

Treatment (P=0.032), timepoint (P<0.001), and their interaction impacted forward activity in the farrowing crate (P<0.001), with CAS-A piglets being more active than CAS piglets at 1-hour post-treatment (P=0.009), and CAS-A tending to be more active than CAS piglets 6 hours post-treatment (P=0.056; Figure 3.3). Treatment (P=0.074) tended to impact vertical activity, while timepoint (P<0.001) and their interaction impacted vertical activity (P<0.001), with the CAS-A piglets being more vertically active compared to CAS piglets at 1-hour post castration (P=0.009; Figure 3.4).

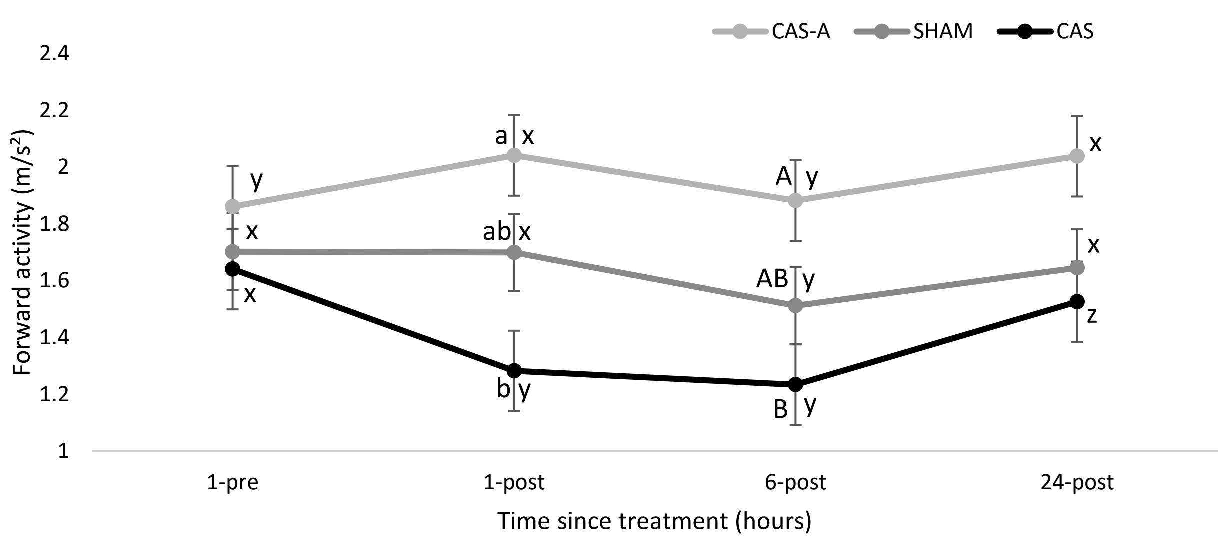

Figure 3.3. Forward activity (lsmean ± SEM; m/s^2) of piglets in their home pen 1-hour prior (1-pre), 1-hour post (1-post), 6-hour post (6-post), and 24-hour post (24-post) castration treatment (CAS: surgically castrated n=21; CAS-A: piglets castrated with analgesics n=20; SHAM: sham-handed piglets n=21). $^{a-b}$ Timepoint means (vertical comparison) with uncommon superscripts differ at P<0.05. $^{A-B}$ Timepoint means (vertical comparison) with uncommon superscripts differ at P<0.10. $^{x-z}$ Treatment means (horizontal comparison) with uncommon superscripts differ at P<0.05.

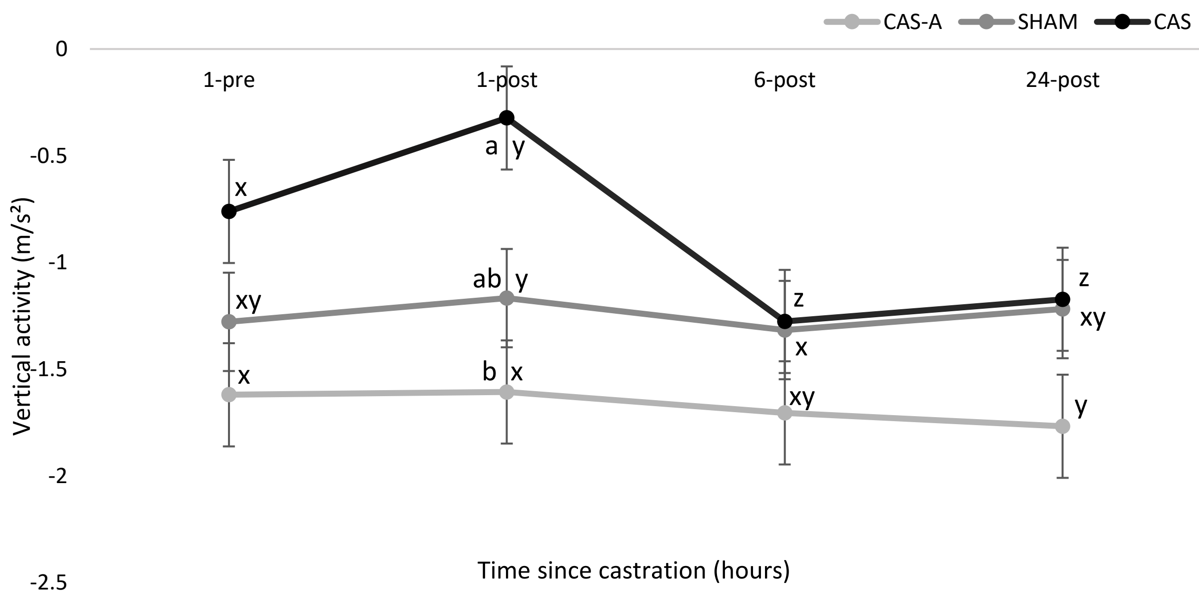

Figure 3.4. Vertical activity (m/s^2 ± SEM) of piglets in their home pen 1-hour prior, 1-hour post, 6-hour post, and 24-hour post castration treatment (CAS: surgically castrated n=21; CAS-A: piglets castrated with analgesics n=20; SHAM: sham-handed piglets n=21). $^{a-b}$ Timepoint means (vertical comparison) with uncommon superscripts differ at P<0.05. $^{x-z}$ Treatment means (horizontal comparison) with uncommon superscripts differ at P<0.05.

Pain Expressions and Affective States

Treatments did not impact total Piglet Grimace Scale scores (P=0.352). Time post-treatment impacted grimace scores (P=0.039), with higher scores at 6 hours post castration compared to 24 hours post castration (Figure 3.5).

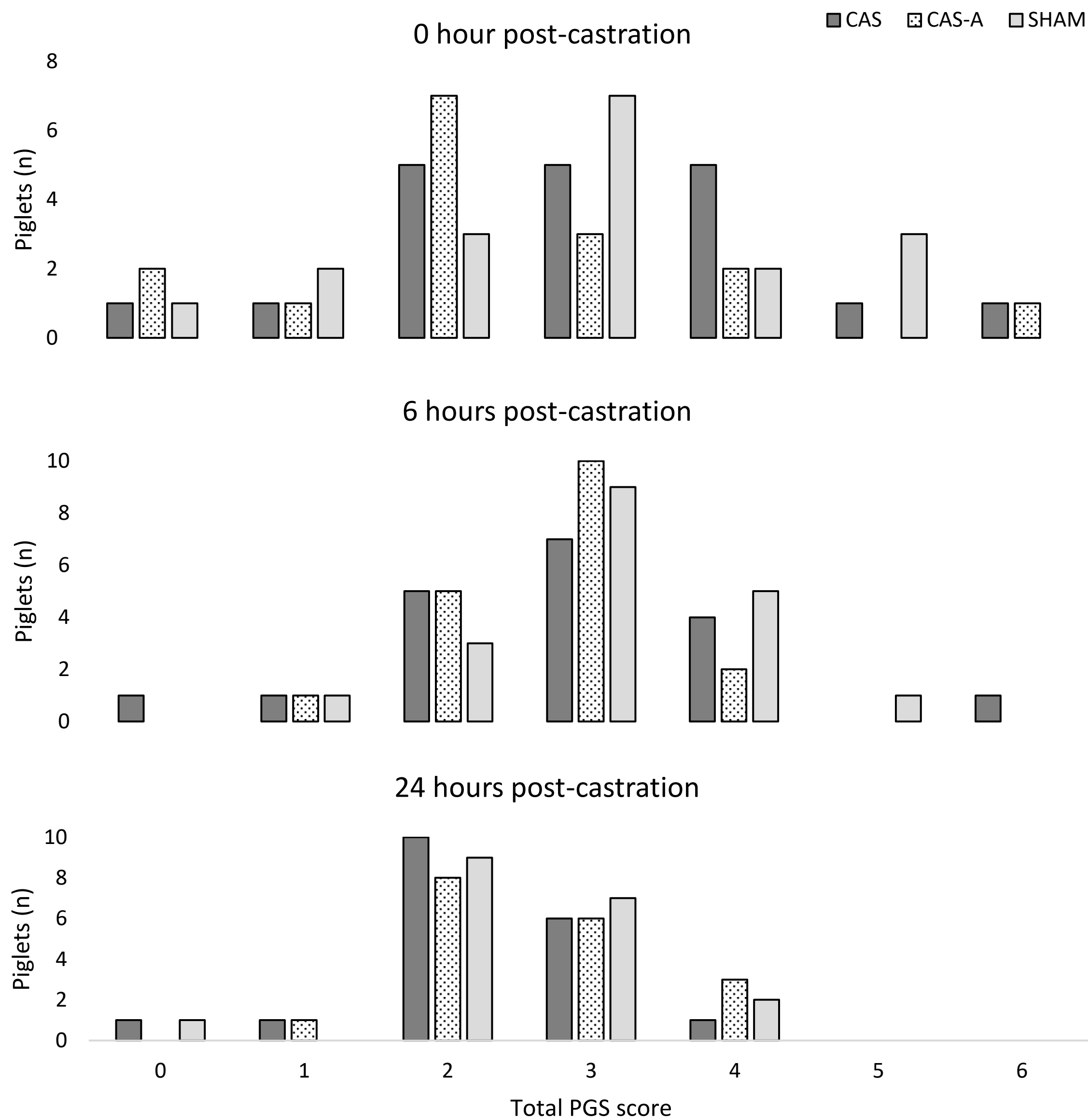

Figure 3.5. Piglet Grimace Scale scores (PGS; 0-6) for castrated piglets (CAS; n=22), piglets castrated with analgesics (CAS-A; n=21), and sham-handed piglets (SHAM; n=22) at 0, 6, and 24 hours post castration.

Castration treatments did not impact tail motions (P=0.229). Tail motion scores differed depending on the time since treatment (P<0.001; Figure 3.6). Most piglets showed a motionless tail immediately after the treatments, followed by a loose wag. More variation in tail motion was observed at 6h and 24h after castration, with most piglets showing a motionless tail or loose wag at 6h, and a loose wag or intense wag at 24h post-treatment. Treatments did not impact tail postures (P=0.201). Tail postures differed depending on the time since treatment (P=0.034; Figure 3.7).

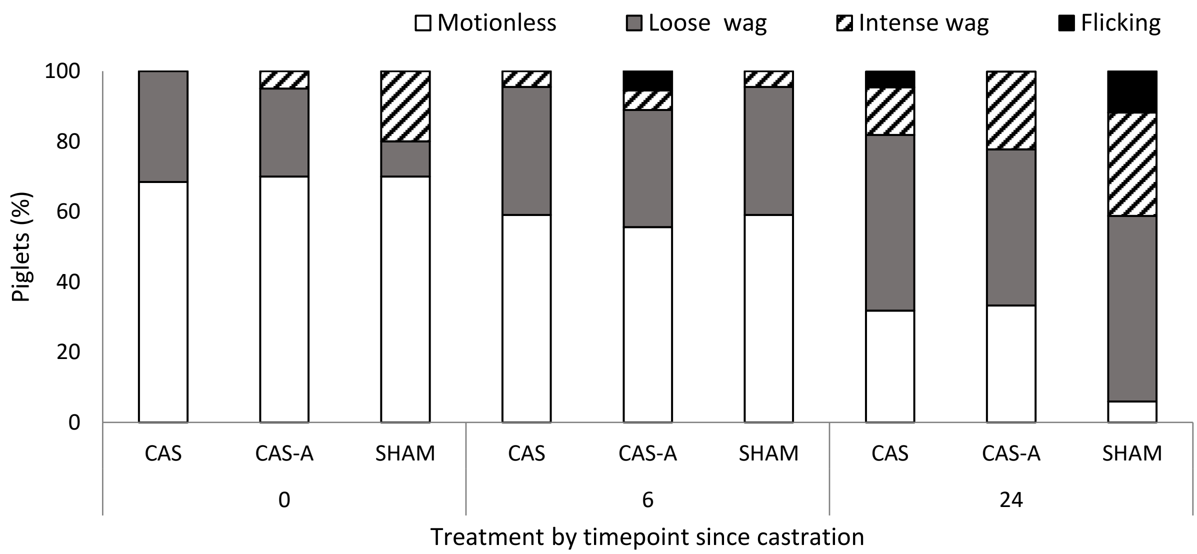

Figure 3.6. Piglets (total %) in each tail motion category by castration treatment (CAS: surgically castrated n=21; CAS-A: piglets castrated with analgesics n=20; SHAM: sham-handed piglets n=21) at 0, 6, and 24 hours since treatments.

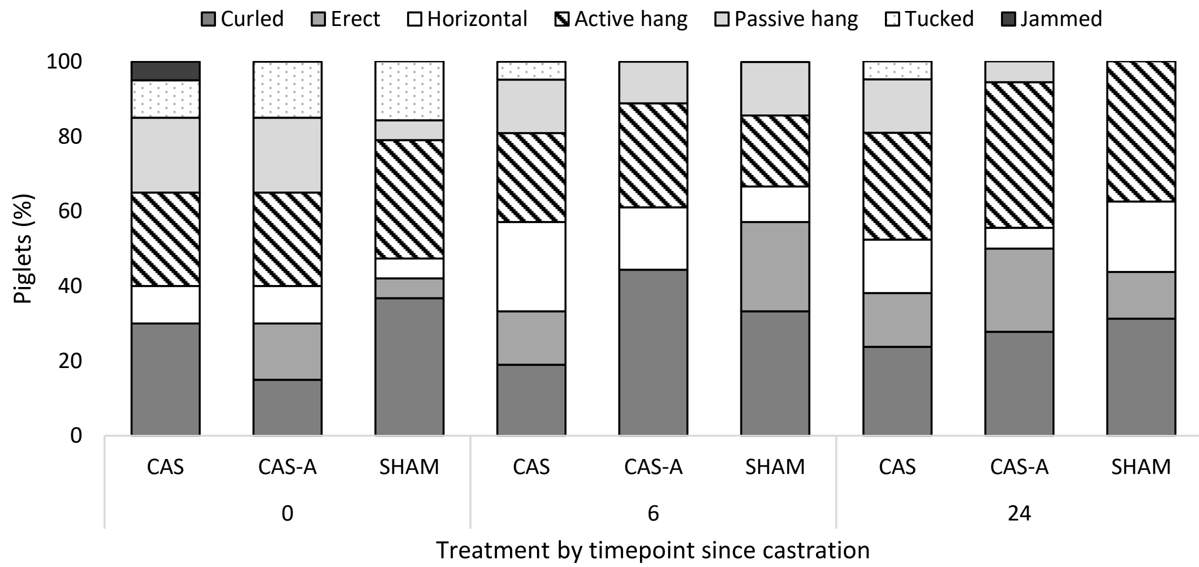

Figure 3.7. Piglets (total %) in each tail posture category by castration treatment (CAS: surgically castrated n=21; CAS-A: piglets castrated with analgesics n=20; SHAM: sham-handed piglets n=21 at 0, 6, and 24 hours since treatments.

3.5.3 Attention Bias Test Data

Activity Measurements

Castration treatment tended to impact forward activity during the attention bias test (P=0.065; Figure 3.8), with more forward activity in CAS compared to CAS-A (P=0.067) piglets, but no difference between CAS and SHAM (P=0.158), or CAS-A and SHAM piglets in WK1 (P=0.150). At WK12, castration treatment did not impact activity during the attention bias test (P=0.650).

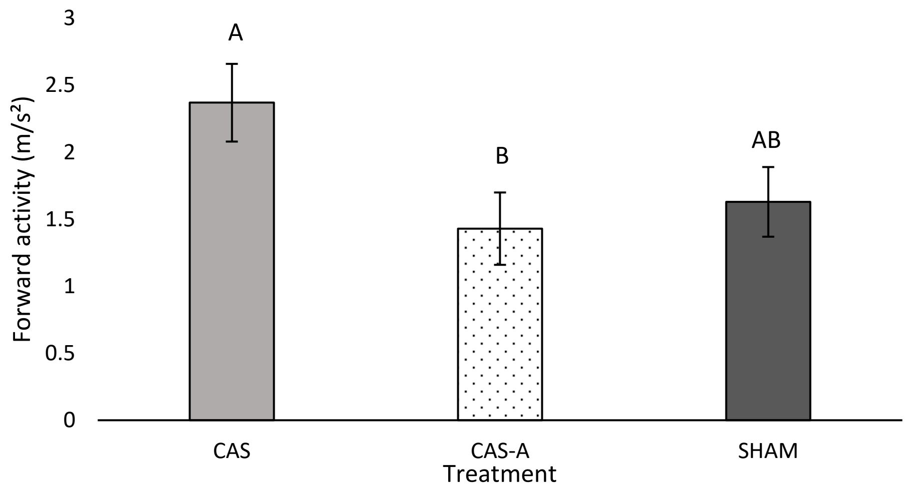

Figure 3.8. Forward activity (LSmeans ± SEM; m/s^2) of piglets during the attention bias test by castration treatment in week 1 (CAS: surgically castrated n=11; CAS-A: piglets castrated with analgesics n=10; SHAM: sham-handed piglets n=11). $^{A-B}$ Means with uncommon superscripts differ at P<0.10.

Attention Bias Test Responses

Pigs' latency to eat did not differ among treatments in WK1 (P=0.131) or WK12; (P=0.479; Table 6). Castration treatment (P= 0.066) and test week (P=0.069) tended to impact the number of pigs eating, but their interaction was non-significant (P=0.258). Fewer CAS (n=3/21) than SHAM piglets (n=9/20) ate in WK1, and fewer pigs ate in WK1 (n = 6/32) compared to WK12 (n=11/29). Castration treatment impacted the time piglets spent eating in WK1 (P=0.020), with SHAM piglets eating longer after the threat was presented than CAS piglets (P=0.014; Table 3.6). Castration treatment tended to impact vigilance behavior in WK1 (P=0.074; Table 3.6), with CAS-A tending to be more vigilant than SHAM (P=0.074). Castration treatments did not impact grunts during the attention bias test for piglets in WK1 (P=0.282) or in WK12 (P=0.162), or high-pitched vocalizations in WK12 (P=0.403).

Table 3.6. Means (± SEM) and p-values of the behaviors displayed after presentation of the threat during the attention bias test at 1 or 12 weeks of age. CAS=surgically castrated; CAS-A=piglets castrated with analgesics; SHAM= sham-handed piglets.

Behavior	Week 1 CAS	CAS-A	SHAM	Treatment effect (p-value)
Latency to eat (sec)	180±6.7	168.5±7.0	160.3±6.7	0.131
Attention to threat (duration in sec)	4.1±0.9	3.9±0.1	2.7±0.9	0.112
Vigilance (duration in sec)	34.5±12.1^{AB}	53.7±12.5^{A}	27.1±12.5^{B}	0.074
Eating (duration in sec)	-0.6±5.3^{b}	8.0±5.6^{ab}	17.7±5.5^{a}	0.020
Exploring (duration in sec)	138.6±12.6	114.8±13.5	117.6±13.2	0.239
	Week 12			
Latency to eat (sec)	142.8±19.7	149.3±19.7	115.8±20.8	0.478
Attention to threat (duration in sec)	3.6±0.7	3.0±0.7	3.4±0.7	0.112
Vigilance (duration in sec)	37.4±22.5	48.9±22.1	32.6±22.9	0.768
Eating (duration in sec)	36.8±14.1	39.1±15.1	64.3±15.7	0.558
Exploring (duration in sec)	113.4±25.2	86.0±24.7	76.5±26.0	0.355

$^{a-b}$ Row means with uncommon superscripts differ at P<0.05.

$^{A-B}$ Row means with uncommon superscripts differ at P<0.10.

3.5 Discussion

This study aimed to determine the impact of a common painful management procedure (surgical castration) on piglet's affective states shortly after the procedure (week 1 of life), and later in life (week 12 of age). Castration treatments impacted both forward and vertical activity levels within the first 24 hours after treatments were applied. While treatment did not impact grimace score or tail measurements, both tended to be impacted or were impacted by timepoint since treatment. Castration treatment tended to impact, or impacted forward activity, number of piglets that ate, and vigilance behavior in week 1 of life. Castration did not impact body weight, vertical activity levels, and all other behaviors displayed during the attention bias test in week 1 of life. When pigs were 12 weeks old, body weight, activity levels, number eating, and behaviors displayed during the attention bias test were not impacted by castration treatments.

Pain experience can reduce piglets' activity (Ison et al. 2016). In our study, CAS piglets' forward and vertical activity in the farrowing crate during the first hour after castration was reduced compared to CAS-A piglets, and forward activity tended to be reduced 6 hours after castration compared to CAS-A piglets. This decrease in activity suggests CAS piglets experienced more acute pain from surgical castration than CAS-A piglets immediately after the procedure, persisting at least 6 hours afterward. The increased forward activity in CAS-A piglets suggests effective acute pain relief from analgesics, although whether it is due to the lidocaine, the flunixin, or both, remains unclear as previous research did not report a consistent benefit for either (Langhoff et al. 2009, Kluivers-Poodt et al. 2013, Burkemper et al. 2020, Merenda et al. 2022). Behavioral observations indicated reduced activity in castrated piglets in other studies (Taylor et al. 2001, Hay et al. 2003, Sutherland et al. 2010, Gottardo et al. 2016, Viscardi et al. 2017). Forward and vertical activity did not differ between treatment groups before the castration procedure or 24 hours afterward, even though the activity was lowest for CAS piglets compared to the other treatment groups throughout. The lack of a difference between treatments 24 hours after the procedures suggests that the acute pain waned in the CAS piglets, with an increase in activity compared to 1 and 6 hours after the procedure. Yet, activity was still reduced compared to before the procedure, suggesting that CAS piglets' activity, thus pain experience, did not fully return to baseline 24 hours after castration and could be indicative of pain lasting longer than 24 hours. Other studies assessing behavioral indicators of pain have found that it can persist anywhere from 2 hours to 4 days post-castration (Taylor et al. 2001, Hay et al. 2003). The lack of difference in forward and vertical activity between CAS and SHAM 1 and 6 hours after castration treatments is unexpected and unclear. Further investigation is necessary to better understand this.

Although the Piglet Grimace Scale was previously validated to detect pain expression after castration (Viscardi et al. 2017), and can be applied successfully by a wide range of stakeholders (Neary et al. 2022b), scores did not differ between castration treatments in the current study. Most piglets had a total score of 2 or 3, reflecting an intermediate pain level that is difficult to interpret (Viscardi et al. 2017). Grimace scores were higher at 6 hours post-treatment compared to 24 hours post-treatment.

Pigs' tail posture can be used as an indicator of tail biting (reviewed by Camerlink and Ursinus, 2020), but also signals emotional experiences unrelated to tail biting (Machado & Iran 2020). In the current study, tails were kept intact, yet tail docking can impact tail postures, motion, and ability to express emotional states (Herskin et al. 2016, Camerlink & Ursinus 2020). Using the circumplex model of affect as described by Camerlink and Ursinus (2020), tail postures and motions can provide insights into emotional valence and arousal at time of assessment (Camerlink & Ursinus 2020). In our study, tail motions and postures were not impacted by castration treatments. Tail postures and motions did differ between sample timepoints. Tail posture and motion have not previously been investigated in the context of piglet castration. A motionless tail, the most commonly observed 'motion' directly after the treatments, is indicative of a low arousal and negative valence, or quadrant 3 in the model of affect, suggesting a negative affective state (Camerlink & Ursinus 2020). Since there was no treatment effect, this may be related to the stress caused by handling regardless of potential pain induced by the treatment. The transition to the loose wag after 6h and 24h represents a shift to a positive valance and positive affective state (Camerlink & Ursinus 2020), suggesting that the potential handling stress had waned. Treatments did not impact tail postures. A jammed tail was observed solely in the CAS treatment at 0 hours post-treatment, albeit by just one piglet, indicating high arousal and negative valence (quadrant 4 in the model of affect), suggesting a negative affective state. Actively hanging or curled tails are the two most common tail motions observed across timepoints, which are indicative of a positive valance. More research is needed to better understand the impact of castration on tail expressions, and how handling could impact those. In the current study tails were kept intact, yet tail docking is a common husbandry practice in the industry.

Attention bias tests can provide insights into animals' affective state by determining anxiety (Crump et al. 2018). The attention bias test results make it difficult to conclusively say that there is increased anxiety in the CAS piglets compared to the other treatments. Forward activity during the attention bias test tended to be higher for the CAS piglets compared to the CAS-A in WK1. This hyperactivity in CAS piglets may indicate more anxiety than in the CAS-A piglets, however, this was only a tendency. This lack of a strong effect could be in part due to the limited sample size. Latency to eat is the most common indicator of anxiety in attention bias tests (Luo et al. 2019, Verbeek et al. 2019, Campbell et al. 2019a, 2022, Anderson et al. 2021). In our study, these latencies did not differ between treatments in WK1 or WK12. This may be due in part to the piglets' interest in the positive stimulus provided, especially in WK1. Previous studies tested piglets after weaning or at 11 weeks of age, also using feed as a positive stimulus (Luo et al. 2019, Ipema et al. 2022). Yet no studies included piglets of pre-weaning age. As piglets solely feed from the sow at WK1 of age, the positive stimulus needed to be liquid. The novelty of feeding from a feeder, and feeding on something other than milk, could have limited their feeding response in this test. Yet, piglets at this age did spend time ingesting the Gatorade, with SHAM piglets spending most time eating compared to the CAS piglets. Further research may be needed to improve piglet responsiveness in the test, for instance by prolonging the test duration to increase the number of piglets eating, especially in WK1. Nevertheless, the results do suggest that piglets may have experienced more anxious affective states in CAS than CAS-A or SHAM, but confirmation is needed.

Treatments did not impact any response variables when piglets were 12 weeks of age. This suggests that any impact that castration had on anxiety was no longer detectable at this age. These results suggest that surgical castration did not have a long-term impact on affective states in the tested piglets.

3.6 Conclusion

Previous research showed that surgical castration causes behavioral and physiological responses indicative of acute and chronic pain, yet the impact of the procedure and associated pain on affective states were not previously studied. The results in the current study suggest that, through a range of different responses for CAS piglets, including reduced activity in the farrowing crate, and a tendency for increased vigilance and hyperactivity during the attention bias test, surgical castration negatively impacted piglets' affective state in the short-term. No evidence was found of a long-term impact on affective states. Furthermore, piglets' activity in the farrowing crate suggests that acute pain was at least partially alleviated in the piglets that received analgesics. Further research could improve the attention bias test design to increase response rates, especially in piglets at a pre-weaning age.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

This work was financially supported by the Virginia Pork Council.

Acknowledgments

We would like to thank Garrett Walker (Virginia Tech) for assistance with animal work, Alyshia Talmage, Ema Hollinger, Jane Perkins, Cody Howard, Kara Crudup, Hailey Roman, and Elise Dow (Virginia Tech) for assisting with data collection and Sadie Grant, Jake Tiss, and Kayleah Corral (Virginia Tech) for assistance with animal care and data collection.

Chapter 4: Recognizing Post-castration Pain in Piglets: A Survey of Swine Industry Stakeholders and the General Public

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Neary, J. M., Porter, N. D., Viscardi, A., & Jacobs, L. (2022). Recognizing post-castration pain in piglets: a survey of swine industry stakeholders and the general public. *Frontiers in Animal Science*, 3:937020. https://doi.org/10.3389/FANIM.2022.937020

4.1 Abstract

We need validated, practical methods for pain assessment in piglets. Pain assessment can help researchers, veterinarians and industry professionals identify the need for analgesia or other pain management approaches when applying painful procedures, such as surgical castration. A pain assessment tool, the Piglet Grimace Scale (PGS), was previously validated in this context, but it is not widely applied. It is important that the PGS can be applied by a range of people, not just pain assessment experts. Our objective was to study the validity and reliability of PGS ratings applied by swine industry professionals and the general public, to assess its potential utility in non-research applications. To do so, we conducted an online Qualtrics survey in which, after completing a brief online training module and a practice test, 119 respondents were asked to rate 9 piglet images showing facial expressions immediately after surgical castration or sham-handling. Respondents were provided information on the castration treatment for each image and had continuous access to the scale throughout the survey. The survey also contained demographic questions. Industry respondents were recruited through networking, and participants from the general public were recruited through Amazon Mechanical Turk. Four trained experts scored each image, and these scores were averaged to produce gold standard scores. Intraclass correlations indicated strong internal consistency among experts, industry and public. ANOVA demonstrated scoring to be moderately comparable between groups. Campbell and Fiske's Multi-Trait Multi-Method framework provided qualified support for the internal validity and reliability of the PGS scale, even applied by non-experts (industry and public). Both response groups were able to recognize pain in castrated piglets. However, public respondents attributed higher levels of pain to sham-handled piglets than industry respondents (2.83 vs. 2.35; p=0.047), and both response groups systematically overestimated pain experience compared to the experts, suggesting more training may be necessary before using the scale in a diagnostic capacity. Nevertheless, overall findings support wide applicability of PGS, even with minimal training, to improve awareness, recognition and monitoring of swine pain among veterinarians, industry professionals and even members of the public.

Keywords: animal welfare, castration, piglet, pain assessment, questionnaire

4.2 Introduction

In the United States (U.S.) swine industry, all piglets undergo invasive husbandry procedures within the first few days of life including vaccines, iron injections, and tail docking. Another invasive procedure is the surgical castration of male piglets intended for slaughter without analgesics or anesthetics (Rault et al. 2011). The American Veterinary Medical Association (AVMA) recommends that after day 14 of age, boars should be castrated using analgesia, anesthesia, or both (AVMA 2020). Typically, piglets are castrated within the first week of life. Therefore, no analgesia or anesthesia is used even though the procedure is painful. Surgical castration can cause acute and chronic pain lasting for 4 days (Hay et al. 2003, Kluivers-Poodt et al. 2013, Ison et al. 2016). Surgical castration involves the manual restraint of the boar, two small incisions or one single horizontal incision on the scrotum, manual removal of testicles, and a cut or tear of the spermatic cord (AVMA 2013). The procedure is typically done by a trained farm technician and takes less than 30 seconds. Piglets are castrated to prevent aggressive behaviors later in life (Rydhmer et al. 2006). Castration also prevents boar taint in the meat, which is caused by the post-pubertal deposition of androsterone and skatole in body fat and causes a foul odor when the meat it prepared (Keenan 2016). Depending on age, breed, and environment, over 50% of intact boars produce pork containing boar taint (Prusa et al. 2011). Seventy-five percent of consumers are sensitive to detect boar taint (Bañón et al. 2004). According to the United States Food Safety and Inspection Service, carcasses that give off a pronounced sexual odor should be condemned (9 CFR 311.20 - Sexual odor of swine. 2012). If the sexual odor is less pronounced, the carcass may be used for comminuted cooked meat products or for rendering. To avoid condemnation, producers will surgically castrate boars to ensure boar taint does not develop.

As the transition from surgical castration to alternative approaches has been slow to non-existent depending on the region, a short-term solution to avoid castration-induced pain would be to provide an analgesic at and after castration. In the U.S., no pharmaceuticals are approved by the U.S. Food and Drug Administration for use in pigs. However, veterinarians can prescribe the extra-label use of anesthetics and analgesics (AVMA 2013), such as non-steroidal anti-inflammatory drugs (i.e. meloxicam) (Viscardi & Turner 2018). In addition, producers can provide analgesics post-castration if pain-related behavior is observed, such as trembling, spasms, scratching, tail wagging and stiffness (Viscardi & Turner 2018). Training producers and industry professionals to assess pain could sensitize them to the potentially frequent expression of pain-related behaviors in piglets and in turn increase willingness to apply analgesics or use alternatives to surgical castration.

A lack of non-invasive, easy-to-use pain assessment tools for the swine industry may hinder pain management. Piglets' pain experience can be determined by quantifying their facial expressions. Species-specific grimace scales are pain assessment tools that could be used as a decision tool for analgesia application and as an indicator of animal welfare (Miller & Leach 2015). A Piglet Grimace Scale was developed based on 10 facial action units (FAUs), which are facial muscle movements that can change in response to pain through regulation of complex limbic systems (Mota-Rojas et al. 2020). When applied, only one FAU related to orbital tightening significantly changed due to tail docking (Di Giminiani et al. 2016). In 2017, another Piglet Grimace Scale (PGS) was developed and validated as a scoring system to recognize pain in piglets after surgical castration (Viscardi et al. 2017, Viscardi & Turner 2018). The PGS is an ordinal scale focusing on expression of ears, cheeks, and eyes, with higher scores representing more severe pain expression. Castrated piglets showed more pain behaviors and had higher PGS scores (more facial grimacing) compared to uncastrated piglets (Viscardi & Turner 2018).

The PGS is not widely applied in the swine industry. This could partly be because of limited knowledge on validity of the tool in a commercial setting. It is important that the PGS can be applied by a range of people with a range of experiences, yet it is unclear whether the PGS has applicability outside of a research context. It is unknown whether the PGS has utility in non-research applications and whether people with (industry professionals) or without animal experience (general public) would be able to provide valid and reliable PGS ratings after minimal training as studies (Viscardi et al. 2017, Viscardi & Turner 2018) suggest the need for training for personnel to properly identify subtle species-specific facial changes. Thus, we aimed to determine whether non-experts would be able to apply the PGS under less-than-ideal conditions with limited training. Through an online survey, the objectives of this study were to 1) evaluate the impact of swine industry experience on survey participants' ability to apply the PGS, and 2) evaluate the ability of all respondents to apply the appropriate PGS scores compared to gold standard ratings by pain experts, using Campbell and Fiske's Multi-Trait Multi-Method framework to assess internal validity and reliability. We hypothesized that industry professionals would rate scenarios differently than the general public, and would show greater consistency with expert raters compared to the general public. In addition, we hypothesized that the internal validity and reliability of thugs, as based on the comparison of non-experts with experts, would be appropriate for use of the tool in the swine industry.

4.3 Methods

All procedures and informed consent protocols were approved by Virginia Tech Human Research Protection Program Institutional Review Board, protocol #20-404. The use of boars in this study was approved by Virginia Tech Institutional Animal Care and Use Committee, protocol #19-288.

4.3.1 Sample

The online Qualtrics survey (SAP, Provo, UT, USA) was distributed (August-September 2020) with the aim to receive responses from experienced swine industry respondents ("industry") and respondents from the general public without swine industry work experience ("public") at a 1:1 ratio. Industry respondents were recruited through Facebook and by direct email to industry stakeholders and university faculty within the authors' network. Facebook posts were not sponsored and were not distributed in any Facebook groups. Facebook users, swine industry contacts and university faculty were invited to disseminate the survey to others with a relevant background, including farm owners, operators, technicians and veterinarians. Simultaneously, public respondents were recruited through Amazon Mechanical Turk (Amazon Web Services, Seattle WA, USA) and received a monetary compensation for their time through the website. Industry respondents did not receive compensation. Inclusion criteria required respondents to be over the age of 18 and living in the U.S. Responses were entered anonymously. We received 129 complete survey responses. Five were omitted because respondents did not live in the U.S., and five were omitted because respondents failed the attention check question. Survey respondents were categorized as either 'public respondents' which was defined as having no professional swine industry experience, or as 'industry respondents' which was defined as having any professional, paid swine industry experience. We included 119 completed surveys in the data analysis, 66 from the public respondents (55%) and 53 from industry respondents (45%).

4.3.1.1 Survey Instrument

The survey consisted of three sections, each described in more detail below: (1) guided training on how to apply the piglet grimace scale (PGS) (Viscardi et al. 2017, Viscardi & Turner 2018), accompanied by 4 practice questions, (2) 9 images of piglets, 6 castrated and 3 sham-handled, to berated using the PGS, and (3) 9 demographic questions. The survey instrument is included in the Appendix A.

4.3.1.2 PGS Training and Practice

The initial section of the survey was aimed at training respondents to apply the scoring system. Training was categorized into topics based on three scoring components; ear positioning (0-3), cheek tightening (0-2), and orbital tightening (0-1) (Viscardi & Turner 2018). After each component was introduced, respondents completed a practice question, after which the correct answer was provided with an explanation. The images used in this practice section were different than those in section 2.

4.3.1.3 PGS Images

The main (second) section of the survey contained nine close-up images of piglets' facial expressions. Images used in this survey were stills taken from videos of nine 7-day old boars from five litters. Within litters, one boar was allocated to the sham castration treatment (SHAM) during which the boar was just handled, and another was allocated to surgical castration treatment (CAS). Individual CAS piglets were held between the handler's legs and surgically castrated by making two vertical incisions on the scrotum and cutting the spermatic cord. Piglets were surgically castrated without anesthesia or analgesia, as is common in the U.S. swine industry (Rault et al. 2011). Piglets were video recorded from the start of restraint, during castration, and for 30s while the piglet was held upright by the body for 30s. A similar recording approach was taken for the SHAM treatment group, but without castration. The boar was restrained between the handler's legs for 30s, followed by holding the piglet up right for another 30s. Nine stills were used in this survey section; six stills from videos of CAS piglets (two during the castration process; four post-castration), and three stills from SHAM handling videos. The number of photos included in the survey were limited because of the negative relationship between completion rate and survey length and question difficulty (Liu & Wronski 2017). Our priority was to determine rater scores for castrated males, therefore those images were over represented compared to sham-handled males. Alongside each image was a short description mentioning either “this piglet was castrated” or “this piglet has not been castrated”. Survey respondents were asked to score each PGS component foreach of the 9 images without a time limit. After each image, respondents were asked to score their level of confidence on a 5-point Likert scale ranging from not confident at all to extremely confident. Respondents had access to a PDF containing the PGS explanation, which they were encouraged to use as a reference throughout the survey. Non-published data from three piglet scenarios (n=119 respondents) showed that falsifying information related to castration status (SHAM for CAS and CAS for SHAM scenarios) did not bias raters’ PGS scores (i.e., raters provided similar scores to the same duplicated piglet scenarios with either SHAM or CAS information).

4.3.1.4 Demographic Questions

The third section of the survey included eleven demographic questions. Respondents were asked about their gender (male, female, non-binary, prefer to self-describe, prefer not to say), age group (18-25, 26-35, 36-45, 46-55, 56-65, 66+ years old), home state or territory, community type (city/urban, suburban, rural, other), meat consumption, pet ownership, and highest level of education. Based on home state or territory entries, respondents were grouped into one of five regions in the U.S. (Northeast, Southeast, Midwest, West, Southwest). Respondents were also asked about any type of professional experience with swine. Survey respondent demographics are compared with U.S. census data in Table 4.1.

Table 4.1. Respondents' demographics (n=119) separated by swine industry experience, with inexperienced respondents (public respondents; n=66) and experienced respondents (industry respondents; n=53). P-values represent the effect of the demographic component (i.e. gender or age) on Piglet Grimace Scale (PGS) scores for the castrated piglet scenarios (CAS) and sham-handled piglet scenarios (SHAM). U.S. census data is presented to compare with survey respondents.

Survey question	Response category	Public respondents (n)[1]	Industry respondents (n)[1]	Total (n)	Total (%)	Census Data (%)	Effect on PGS scores (P-value)	
							CAS	SHAM
Gender	Male	35	27	62	52	49	0.472	0.950
	Female	30	26	56	47	51		
	Prefer not to say	1	0	1	1			
Age[2]	18-25	14	12	26	22	9	0.830	0.131
	26-35	23	19	42	35	14		
	36-45	18	15	33	28	13		
	46-55	6	4	10	8	12		
	56-65	4	3	7	6	13		
	66+	1	0	1	1	11		
United States region of residency	Northeast	15	4	19	16	17	0.700	0.712
	Southeast	5	4	9	8	28		
	Midwest	21	27	48	40	21		
	West	20	16	36	30	21		
	Southwest	5	2	7	6	13		
Community Type	City/Urban	24	16	40	34	71	0.297	0.264
	Suburban	29	11	40	34	10		
	Rural	13	26	39	33	19		
Education Level	Less than a high school degree	0	0	0	0	10	0.681	0.414
	High school degree or equivalent (GED)	4	1	5	4	28		

	Some college (no degree)	14	0	14	12	17		
	2-year degree	6	1	7	6	10		
	4-year degree (bachelors)	24	26	50	42	22		
	Master's degree	10	11	21	18	9		
	Professional degree	4	4	8	8	1		
	Doctorate (PhD)	4	10	14	12	2		
Meat consumption	Yes	57	52	109	92		0.443	0.109
	No	9	1	10	8			
Pet ownership	Yes	49	47	96	81		0.395	0.802
	No	17	6	23	19			

[1] Respondent categorization into "general public" or "industry" was based on respondent's answer to the question: Do you have any professional work experience with swine and the swine industry?

[2] The age ranges for the census data were <18, 18-24, 25-34, 35-44, 45-54, 55-64, and 64+. Census Data from the <18 category is not presented.

4.3.2 Data Processing and Analysis

Data processing and primary analysis was conducted in R (v4.1.2; R Core Team 2021), with additional Multi-Trait Multi-Method analysis in Microsoft Excel. Code and anonymized data can be found in (Neary et al. 2022a).

4.3.2.1 Gold Standard Score

We calculated a gold standard PGS score as standard of comparison for each of the 9 images. Four trained researchers were blinded to treatment and provided scores for the same nine piglet scenarios. Averaged two-way random-effects Intraclass Correlation Coefficients (ICC) were calculated with the ICC function in the psych package (v2.1.9; Revelle 2021) for eye, ear and cheek scores, and based on guidelines from (Koo & Li 2016), showed a good (0.79, 95% C.I. = 0.53-0.93) ICC for ears, excellent (0.94, 95% C.I. = 0.87-0.98) ICC for cheek, and perfect (1.0, C.I. not applicable) ICC for eye scores. These four ratings were averaged to calculate a standard of comparison (gold standard) for each component of each image.

4.3.2.2 Reliability

Inter-group reliability measures the consistency of ratings across different groups. One-way ANOVA between the three respondent groups was conducted on total PGS scores, with post-hoc analysis of group differences conducted using Tukey's HSD when the F-tests was significant at the $p<0.05$ level. Analysis was conducted separately for CAS and SHAM images, as well as in aggregate, using the aov and Tukey HSD functions in the stats package (v4.1.2; R Core Team 2021) in R. Significant ANOVA F-tests indicate cross-group variation in mean total scores for the same scenario. Significant Tukey's HSD tests indicate a specific group's mean ratings were significantly higher than another after adjusting standard errors to a family-wise $p<0.05$.

Intra-group reliability measures the consistency of ratings across independent raters from the same background (expert, industry or public) and was calculated using ICC2 and ICC2k Intraclass Correlation tests (Howell 2018) with the ICC function in the psych package (v2.1.9; Revelle 2021). Both measures have a 0-1 range. High ICC2 values indicate two random individuals from the group are likely to score the given components (ear, cheek, eye, and total PGS score) similarly. ICC2k is a parallel measure but assesses the likely consistency of the mean ratings in each group with different samples.

4.3.2.3 Convergent and Discriminant Validity

Validity was assessed using Campbell and Fiske's Multi-Trait Multi-Method (MTMM) model (Campbell & Fiske 1959). Most common measures of reliability and validity, such as Cronbach's Alpha (Cronbach 1951), are what Campbell and Fiske term "convergent" tests of observed correlation of a set of items. While they attempt to assess the degree to which items measure a single underlying construct (like piglet grimacing), convergent measures are unable to discriminate between shared true variance (construct validity) and shared error variance, such as correlation due to the method of data collection (Alwin 2005). Campbell and Fiske argued that "tests can [also] be invalidated by too high correlations with other tests from which they were intended to differ" (Campbell & Fiske 1959 p. 81). That is, ideally, measures of the same underlying trait should not only closely resemble each other (convergent validity) but also differ meaningfully from measures of other traits (discriminant validity). For example, if the PGS is performing well, we should expect different raters to rate the same images more similarly than the same raters rating different images, because different images represent different actual piglet experiences. If all criteria are met, MTMM provides more robust evidence of reliability and validity. If some are not met, MTMM can help identify whether validity is impaired primarily by individual raters, images or features or whether there more complex interactions to address.

MTMM was proposed to simultaneously test both convergent and discriminant validity using data collected on multiple distinct traits, but with each trait measured using the same variety of multiple methods (Alwin 2007). Methods may vary in many ways, such as question wording, number of response categories, or time of data collection (e.g., longitudinal). In MTMM, a correlation matrix of each trait as measured within and between each method is used to evaluate four specific criteria, one for convergent validity and three for discriminant validity. The matrix is divided into blocks, with each block containing the correlations of each trait as measured by two methods, as in the 3-trait, 3-method example in Table 4.2. Based on the assumption that items measuring the same trait or using the same methods should be more closely correlated than others, (Campbell & Fiske 1959) propose the following four validity criteria (CF1-CF4):

1. Correlations between different methods measuring the same trait (bolded in Table 1, also called the validity diagonal) should be large enough to motivate investigating validity further.
2. Correlations in the validity diagonal should be higher than others in the same row or column of the same block.
3. The correlation between two methods of measuring the same trait should be higher than between two traits measured with the same methods.
4. Patterns of heterotrait correlations (non-1, non-bolded in Table 1) should be similar between all monomethod blocks (method 1-1 and method 3-3 in Table 1) and between all heteromethod blocks (method 1-2 and method 2-3 in Table 1).

Table 4.2. Example multitrait-multimethod Correlation Matrix. Bolded values indicate the validity diagonal (correlations between different methods measuring the same trait).

Method	Trait	Method 1			Method 2			Method 3		
		X	**Y**	**Z**	**X**	**Y**	**Z**	**X**	**Y**	**Z**
1	X	1								
	Y	r(x1,y1)	1							
	Z	r(x1,z1)	r(y1,z1)	1						
2	X	**r(x1,x2)**	r(y1,x2)	r(z1,x2)						
	Y	r(x1,y2)	**r(y1,y2)**	r(z1,y2)						
	Z	r(x1,z2)	r(y1,z2)	**r(z1,z2)**						
3	X				**r(x2,x3)**	r(y2,x3)	r(z2,x3)	1		
	Y				r(x2,y3)	**r(y2,y3)**	r(z2,y3)	r(x3,y3)	1	
	Z				r(x2,z3)	r(y2,z3)	**r(z2,z3)**	r(x3,z3)	r(y3,z3)	1

The substantive meaning of each criterion is discussed below, along with evaluation methods. Polychoric correlations calculated with the polychoric function and significance calculated with the corci function (psych package, (Revelle 2022)) are used to estimate relationships between items with ordinal measurement (PGS components) on a continuous latent scale. Each image is treated as a trait on the assumption that the piglets have unique pain experiences and patterns of grimace response expression. PGS components (eye, cheek, ear) are treated as different methods of measuring the same underlying concept of piglet pain (Viscardi & Turner 2018).

CF1 measures convergent validity and establishes that multiple measures of the same trait capture a similar underlying concept. The remaining criteria all test divergent validity. To test CF1 for each correlation, we will use the following categories: non-significant, low (<0.3), moderate (0.3 to less than 0.6), and high (>0.6). Both significance and strength are relevant to establishing baseline convergent validity.

The remaining criteria measure different aspects of divergent validity. CF2 establishes whether the results of two ways to measure the same trait resemble each other more closely than the same methods used to measure two different traits, e.g. whether the traits are meaningfully different. This is analyzed in summary using a T-test of the difference in mean correlation between monotrait-heteromethod (MTHM) and heterotrait-heteromethod (HTHM) cells, as well as by descriptive analysis of the observed proportion of HTHM correlations in the same row and column that are less than the MTHM.

CF3 tests if relationships between items are driven primarily by substantive (trait) or technical (method) differences. This is evaluated with a T-test of difference of means between MTHM and heterotrait-monomethod (HTMM) cells.

CF4 tests whether traits demonstrate similar patterns of relationship when measured in different ways (including both individual measures and composites from multiple methods). This is tested using pairwise between-block correlations of all heterotrait (HT) cells.

In summary, we use MTMM to address the limitations of common summary statistics for reliability (i.e., Alpha, ICC), which only measure internal convergent validity – whether multiple items or raters to covary closely – and thus are unable to distinguish between substantive relationships and common method or error variance. The first validity criterion (CF1) accomplishes the same goal as traditional reliability measures, but the remaining three criteria provide more granularity by assessing discriminant validity; e.g., the extent to which measures that ought to (theoretically) be different do in fact differ. This in turn can help discern the differences between rater bias and poor performance on particular features or images.

4.4 Results

4.4.1 Overall Scores and Intergroup Reliability

Figure 4.1 summarizes mean PGS scores for each group of raters by PGS component (ear, cheek, eye) and treatment (castration or sham-handled) in a table, alongside a Forestplot (R forestplot package v2.0.1, Gordon & Lumley 2021) of public and industry PGS means with 95% confidence intervals, centered on the gold standard ratings established by experts. The mean total PGS score across all raters and images was 3.09, compared to the mean gold standard of 2.50, with 3 being the most common PGS score and 18% falling into the "no- to low pain" category (PGS=0-1) compared to 14% in the gold standard group, following Viscardi and colleagues' (Viscardi et al. 2017) categorization. Overall, respondents indicated a confidence level between 3 and 4, indicating feeling 'somewhat confident' in their scenario scores.

Component	Treatment	Gold Standard	Public	Industry
Cheek	Castration	0.92	1.17	1.24
Cheek	Sham	1.42	1.25	1.07
Ear	Castration	1.04	1.54	1.61
Ear	Sham	1.17	1.29	1.09
Eye	Castration	0.50	0.57	0.60
Eye	Sham	0.00	0.30	0.19
Total	Castration	2.46	3.28	3.45
Total	Sham	2.58	2.83	2.35

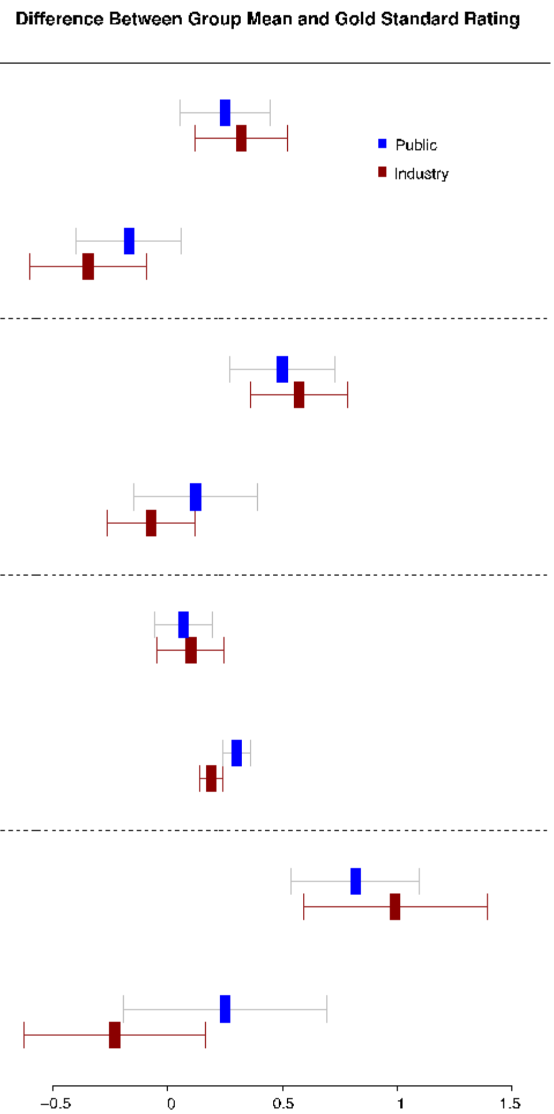

Figure 4.1. Respondents' scores (general public and industry respondents) by feature (eye, ear, cheek) and treatment (castration or sham-handled) relative to gold standard scores.

One-way ANOVA (Table 2) indicated no significant differences in total PGS score between the three rater groups across all images (p=0.266). However, differences were significant within the subset of CAS images (p=0.034) and tended to be significant (p=0.060) for SHAM images. Using post-hoc comparison with Tukey's HSD (Table 4.3), a mean score difference was found for CAS images of 0.991 between industry and experts (p=0.034) and a marginal score difference of 0.817

between public and experts (p=0.095). For SHAM scenarios, a mean score difference of 0.481 was found between public and industry (p=0.047).

Table 4.3. One-Way ANOVA of Piglet Grimace Scale (PGS) mean scores as a function of Rater Group by treatment with Tukey's HSD post-hoc comparisons of * significant (p<0.05) and +marginally significant (p<0.10) results.

One-way ANOVA of PGS on Rater Group						
Scenario		**Degrees of Freedom**	**Sum-of-squares**	**Mean squares**	**F**	**Pr(>F)**
All images		2	1.49	0.745	1.339	0.266
Castration		2	3.99	1.996	3.472	0.034*
Sham-handled		2	6.81	3.405	2.873	0.060+

Tukey's HSD Comparison of PGS Means					
Scenario	**Rater Comparison**	**Difference**	**95% Confidence Interval**		**Adjusted Pr(>T)**
			Lower	**Upper**	
Castration	Industry-Expert	0.991	0.058	1.924	0.034*
	Public-Expert	0.817	-0.110	1.743	0.095+
	Public-Industry	-0.174	-0.506	0.157	0.428
Sham-handled	Industry-Expert	-0.231	-1.571	1.109	0.912
	Public-Expert	0.250	-1.080	1.580	0.896
	Public-Industry	0.481	0.005	0.957	0.047*

4.4.2 Intragroup Reliability

The Intraclass Correlation results can be found in Table 4.4. Within-group consistency of estimates of the mean (ICC2k) was high across all groups and features, with ICC2k for the total score ranging from 0.76 (experts) to 0.96 (industry). The ICC2 reliabilities of individual ratings (as compared to means) were lower and more variable, with public ICC2 ranging from 0.14 to 0.25, industry from 0.30 to 0.39, and experts from 0.44 to 1. The ordering of expert-industry-public from most to least reliable was consistent across all components for ICC2.

Table 4.5. Average and Single-Rater Intraclass Correlation (ICC) with 95% Confidence Intervals (CI) by Piglet Grimace Scale (PGS) feature and total scores. * All observed ratings identical; no confidence interval can be calculated. N/A, not applicable.

		ICC2k (average)			ICC2 (single-rater)		
Feature		**Public**	**Industry**	**Experts**	**Public**	**Industry**	**Experts**
Ear	Intraclass Correlation	0.94	0.97	0.79	0.2	0.39	0.48
	95% Confidence Interval	(0.89-0.98)	(0.94-0.99)	(0.53-0.93)	(0.11-0.43)	(0.24-0.66)	(0.22-0.77)
Cheek	Intraclass Correlation	0.95	0.96	0.94	0.22	0.33	0.81
	95% Confidence Interval	(0.90-0.98)	(0.93-0.99)	(0.87-0.98)	(0.12-0.46)	(0.20-0.60)	(0.63-0.93)
Eye	Intraclass Correlation	0.96	0.96	1	0.25	0.3	1
	95% Confidence Interval	(0.91-0.98)	(0.92-0.99)	NA*	(0.14-0.50)	(0.17-0.56)	NA*
Total	Intraclass Correlation	0.91	0.96	0.76	0.14	0.33	0.44
	95% Confidence Interval	(0.83-0.97)	(0.93-0.99)	(0.46-0.92)	(0.07-0.33)	(0.20-0.60)	(0.18-0.78)
N		66	53	4	66	53	4

4.4.3 Convergent and Discriminant Validity

The full MTMM matrix of correlations between image and component ratings across all raters is shown in Table S2. Findings for validity criteria are addressed separately below.

4.4.3.1 CF1: Correlations between different methods measuring the same trait (bold in Table 4.2, also called the validity diagonal) should be large enough to motivate investigating validity further.

There are three heteromethod combinations (ear-cheek, ear-eye, cheek-eye), each measured on 9 scenarios, with polychoric correlations in Table 4. Two-thirds (18/27) of correlations are statistically significant, with 44.4% (12/27) of correlations showing a moderate magnitude of at least 0.3. No correlations had a high magnitude. Overall, ear and eye ratings were less correlated than the other combinations, with only two of nine correlations showing a magnitude of at least 0.3 (Table 4.5).

Table 4.5. MTMM Monotrait Heteromethod Correlations for all PGS raters (n=123) per scenario (* = polychoric correlation significantly different from 0, p<0.05; bolded = magnitude > 0.3).

Scenario	Ear-Cheek	Ear-Eye	Cheek-Eye
1	0.034	0.264*	0.129
2	0.243*	0.161	0.033
3	**0.348***	-0.081	**0.318***
4	**0.541***	0.242*	**0.448***
5	**0.312***	**0.470***	0.174
6	**0.443***	0.289*	0.287*
7	0.185*	0.130	**0.422***
8	**0.473***	**0.336***	**0.319***
9	0.165	0.104	**0.346***

4.4.3.2 CF2: Correlations in the validity diagonal should be higher than others in the same row or column of the same block.

This test compares the MTHM correlations in Table 4 to correlations using the same pair of methods (i.e., ear and cheek) but with two different images (HTHM). The overall mean MTHM correlation of 0.266 is significantly higher than the mean HTHM correlation of 0.078 (difference=0.187, T=5.279, n=133, p<0.001). More than 3/4 of individual cells met criterion CF2 in every heteromethod block (ear-cheek: 0.833, ear-eye: 0.833, cheek-eye: 0.764). None of the 9 images failed the criteria in more than ten of the 24 comparisons. This indicates that different facial measures of pain in the same piglet are more closely related than those measures across different piglets.

4.4.3.3 CF3: The correlation between two methods of measuring the same trait should be higher than between two traits measured with the same methods.

Although the observed mean of MTHM correlations (0.266) is marginally higher than that of HTMM cells (0.244), the difference of 0.022 is not statistically significant (T=0.4268, n=133, p=0.6702). Criterion CF3 is not met and we find no evidence that differences between methods (facial feature ratings) by trait (scenario) were smaller than differences between separate scenarios when measuring with the same rating component.

4.4.3.4 CF4: Patterns of heterotrait correlations should be similar between all monomethod and between all heteromethod blocks.

CF4 is tested using the correlation of the vectors of all heterotrait cells between each pair of heteromethod blocks and between each pair of monomethod blocks. Correlations were significant and positive ($p<0.05$) for 3 of the 6 combinations (Table 4.6). All non-significant correlations either included eye-eye or eye in both blocks, implying eye grimace ratings were not as closely correlated to other measures as ear and cheek were to each other.

Table 4.6. MTMM Heterotrait Inter-block Correlations (bold = $p<0.05$)

Block 1	Block 2	Polychoric Correlation (r)	t Statistic	Degrees of Freedom	Significance (p)
Ear-Ear	**Cheek-Cheek**	**0.429**	**2.576**	**34**	**0.015**
Ear-Ear	Eye-Eye	0.193	1.727	34	0.093
Cheek-Cheek	Eye-Eye	0.178	0.693	34	0.493
Ear-Cheek	**Ear-Eye**	**0.286**	**2.691**	**70**	**0.009**
Ear-Cheek	**Cheek-Eye**	**0.293**	**2.615**	**70**	**0.011**
Ear-Eye	Cheek-Eye	-0.100	-0.207	70	0.837

4.5 Discussion

Pain associated with surgical castration is a welfare concern for piglets, and most, if not all male piglets intended for meat are surgically castrated without analgesia in the U.S. (Rault et al. 2011). Pain assessment using the PGS could be a quickly applicable tool to make decisions about pain alleviation during or after the procedure. This is the first survey to investigate aspects of the applicability of the PGS in the U.S. swine industry. Using an online survey with industry and public respondents, we found that respondents showed a consistent ability to score pain expression in castrated piglets. Yet, public respondents attributed higher levels of pain to sham-handled piglets than industry respondents, and both overestimated pain compared to experts. Campbell and Fiske's Multi-Trait Multi-Method framework provided qualified support for the internal validity and reliability of the PGS scale, even applied by non-experts after minimal training.

Comparing the 119 survey respondents to the 2019 American Community Survey estimates for the U.S. population (United States Census Bureau 2019), the gender distribution of respondents did not differ significantly from the population, but respondents were younger, more often located in suburban or rural communities, and more highly educated (Table S1). In terms of region, survey respondents were mostly from the West and Midwest (30% and 40% respectively), which were overrepresented compared to the Southeast (8%) and Southwest (6%) (United States Census Bureau 2019). The skew towards the West and the Midwest is likely due to the concentration of swine production in those regions (NASS 2016). The skewed sample in this study may limit the possibility to extrapolate results but does provide a first insight in the ability of respondents to apply the PGS.

PGS scores were predominantly in the “unclear” or “moderate to high” pain ranges for both castrated and sham-handled piglets. Echoing previous findings, expert scores did not differ between castrated and sham-handled piglets (di Giminiani et al. 2016, Viscardi & Turner 2018). Thus, while PGS has been found to covary with other indicators of pain (Viscardi et al. 2017, Viscardi & Turner 2018, Vitali et al. 2020, Vullo et al. 2020) and several studies have shown that surgical castration causes acute pain in piglets (Hay et al. 2003, Kluivers-Poodt et al. 2013, Ison et al. 2016), the scale's poor discrimination at moderate pain levels may limit its diagnostic value for researchers and veterinarians without additional measurements. This is consistent with prior findings on facial grimace scales (Vullo et al. 2020, Hernández-Avalos et al. 2021). For application of a dog grimace scale, evaluator age, experience, training, and image quality impacted the way evaluators interpreted facial changes (Barletta et al. 2016). Additionally, the accuracy of these scales can be affected by the type of pain the animal is experiencing, as well as the way that animal is coping with the pain (Mich & Hellyer 2008). Nevertheless, the PGS' low-cost and non-invasive nature position it well for use in training and sensitization of industry professionals, as well as for initial identification of animals who may require closer attention or observation in relation to painful procedures. Additional measurements, such as physiological responses (i.e., heart rate, respiratory rate) and behavioral responses (i.e., isolation and trembling) can further assist in pain assessment (Viscardi & Turner 2018, Hernández-Avalos et al. 2021).

Experts applied the scale more consistently than individuals in industry or the public (ICC2) but estimated means were reliable within every group (ICC2k). While both industry and public raters scored CAS images higher than gold standards, they diverged on SHAM images, with industry rating them lower than the public. Without blinding or deception to CAS/SHAM treatment, we cannot distinguish the extent to which such differences relate to priming of respondents with the treatment type (as opposed to general differences in sensitivity). Increasing the number of images may have improved the reliability (ICC2) by reducing the influence of individual outliers, but we sought to test how well the PGS performed under less-than-ideal conditions (non-expert raters with minimal training and a limited number of images). Like experts, survey respondents provided mean PGS scores in the difficult-to-interpret intermediate range of pain expression (Viscardi et al. 2017).

SHAM PGS scores were unexpectedly high, as piglets were only handled so should not experience pain. We theorize that facial expression associated with vocalizations in response to handling may have impacted these outcomes. The developers of the PGS also theorized that exposure to livestock (animal experience) contributed to differences in scores (Viscardi & Turner 2018), and this is supported by the difference in scores between public and industry respondents in the current study. While this suggests PGS may not be fully comparable between raters with different experience levels, the strong internal consistency means it can still be reliably used for training veterinary or industry professionals and sensitizing professionals and the public to pain in swine, helping to dispel persistent misconceptions (Steagall et al. 2021) and providing a rapid, simple and unobtrusive evaluation tool that can be more easily applied than behavioral or biomarker scales (Leach et al. 2012, Vitali et al. 2020, Schmid & Steinhoff-Wagner 2022).

The MTMM findings provide additional validation and insight into the validity and reliability of different aspects of the PGS. Results for CF1 showed ear, cheek and eye grimace measures to be meaningfully correlated, but not very highly. Differences between images (CF2) and facial components (CF3) were both frequently significant and virtually identical in overall magnitude. However, this failure to establish divergent validity for criteria CF3 may be related to our design, where neither traits nor methods are characteristics of the rater as compared to a traditional survey MTMM, where traits of respondents are measured. Rather, in our study “traits” were the 9 scenarios, not the respondents, and “methods” were the facial features that were scored. Results for CF4 were mixed. The prominence of “eye” in nonsignificant relationships in CF4 and elsewhere (e.g. the ear-eye column in CF1) despite its strong internal consistency (high ICC for all groups) suggests that measurement differences such as the range (e.g. using a 2-point instead of 3- or 4-point subscale) may be producing weaker relationships with other scale components.

Overall, by using a more comprehensive range of measures of internal validity and reliability, our findings complement and support studies that emphasized compatibility of PGS with other indicators of pain. Additionally, by providing only brief training and including raters with a wide range of experience, this study expands the range of potential applications of PGS in less-than-ideal circumstances, such as training and sensitizing new veterinary and industry professionals. Differences between non-expert and expert scores may still be related to level of PGS training. The initial PGS explanation and training were designed to be short (approximately 5 minutes) to ensure respondents would complete the survey. Continuous access to the explanatory PDF was theorized to reduce the training requirement. Extensive training can improve inter- and intra-rater consistency as shown for a rat grimace scale(Zhang et al. 2019), and 30-min in-person training improved inter-rater agreement for assessors using a horse grimace scale (Dai et al. 2020). Even though training was limited in the current study, agreement between respondent groups were high, with no overall ANOVA differences. A supplemental question on respondents’ self-reported confidence levels indicated they felt ‘somewhat confident’, suggesting that respondents felt adequately trained, with industry respondents slightly more confident than public respondents. Formal training methods have not been tested for the PGS (Viscardi et al. 2017, Viscardi & Turner 2018), and further research should elucidate the required level of training for reliable application.

Providing short videos or multiple images of the same piglet at different angles, or using methods like MTMM to pre-validate training images that perform well may be helpful in order to more accurately score grimaces. Future studies should also attempt to improve understanding of pain and consistency of ratings at moderate levels and investigate sources of inter-group differences in ratings. In improving research on swine pain treatment, applying more holistic measurement models like MTMM in studies featuring additional indicators (behavioral, biomarker, etc.) can improve understanding of inconsistent findings related to pain caused by invasive procedures, as well as treatment effectiveness.

While mixed findings may require discussions about the value of PGS as an assessment tool for pain reduction strategies, at least used in isolation, this study suggests a wider range of potential applications. These include use in veterinary training and practice to dispel misunderstandings about animal pain tolerance (Steagall et al. 2021), practice of visual recognition of pain cues, use in industry to sensitize professionals to the need for pain control, help them recognize signs of distress, and even a potential use in public education about animal welfare. Artificial intelligence and machine learning models can also help, both to improve the quality of images used in training and potentially to enable automated early identification of potential distress in large-scale farms (Steagall et al. 2021).

4.6 Conclusion

In conclusion, through an online survey we evaluated the impact of swine industry experience on survey participants' ability to apply the Piglet Grimace Scale to pictures of surgically castrated and sham-handled piglets. In addition, we tested multiple aspects of internal validity and reliability of the PGS, as applied by non-experts compared to experts. Survey respondents showed consistent pain expression scores, yet agreement with gold standard scores was limited. Longer, more in-depth training may be useful, especially for those who lack animal experience. Overall, we conclude that the Piglet Grimace Scale can be reliably applied in a castration context by both industry professionals and members of the public that receive limited training. However, calibration with expert scores may be needed to avoid systematic overestimation of the pain experience in recently-castrated piglets.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

JMN and LJ contributed to conception and design of the study. JMN conducted the animal-related work. JMN, LJ and NDP contributed to the design of the survey. JMN and LJ organized distribution of the survey. JMN, LJ and NDP collected expert score data. AVV contributed to expert score data. NDP performed data analysis. JMN wrote the first draft of the manuscript. LJ and NDP wrote sections of the manuscript. AVV, LJ and NDP contributed to manuscript revision, and all authors read, and approved the submitted version.

Funding

This work was supported by the USDA National Institute of Food and Agriculture, Hatch Multistate project 1024623.

Acknowledgments

We would like to thank Sadie Grant and Phil Hollingshead (Virginia Tech) for assistance with animal work and Kara Crudup and Cody Howard (Virginia Tech) for assisting with data collection.

Chapter 5: Pilot study: Public and industry knowledge and perceptions of U.S. swine industry castration practices

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5.1 Abstract

In the United States (U.S.), surgical castration of male piglets is typically performed without any form of analgesia. This may raise concerns with the public; however, little is little known about current public knowledge on swine industry practices. In this pilot study we aimed to gain initial insight into public knowledge and perception on castration and analgesia use and compared this to industry stakeholders. Through an online survey, 119 respondents were asked 4 questions about castration in the U.S. swine industry. Industry respondents were contacted via social media and networking, the general public through Mechanical Turk. Survey responses were categorized by experience (industry vs. public). Industry respondents were more aware of surgical castration, and lack of analgesia use compared to the general public. Respondents from rural communities and those with more education were more aware of castration practices. Those from rural communities were more aware of the lack of analgesia use than respondents from urban or suburban communities. Based on the results from this small initial sample, knowledge on industry practices was especially lacking for public respondents, but also for a minority of industry respondents, indicating opportunities for education and further research on the topic.

Keywords: animal welfare; surgical castration; consumer and citizen perception; piglet; questionnaire; swine industry.

5.2 Introduction

Male piglets (*Sus scrofa domesticus*) intended for slaughter are surgically castrated in the United States (U.S.) swine industry. The procedure is performed without analgesics or anaesthetics and involves manual restraint of the boar, two incisions on the scrotum, removal of the testicles, and a cut or tear of the spermatic cord (American Veterinary Medical Association Welfare Division 2013). Castrations are performed by a trained technician and can take approximately 30 seconds. Castration is performed to prevent aggressive behaviours later in life (Rydhmer et al. 2006), and to prevent boar taint in pork (American Veterinary Medical Association Welfare Division 2013). Boar taint is caused by the post-pubertal deposition of androsterone and skatole in body fat, causing the meat to have an abnormal and somewhat foul odour when cooked (Keenan 2016). Prevalence of boar taint in pork ranges from 10-50% depending on factors such as age, breed, and environment (Prusa et al. 2011, Aluwé et al. 2015, Channon et al. 2018). Consumer sensitivity to boar taint ranges from 11-75% depending on factors like age, gender, and geographic location (Bañón et al. 2004, Blanch et al. 2012, Channon et al. 2018). According to the U.S. Food Safety and Inspection Service, carcasses with boar taint should be condemned (9 CFR 311.20 - Sexual odor of swine. 2012). Producers surgically castrate boars to ensure boar taint does not develop and to avoid subsequent condemnation.

Public awareness of castration practices could incentivize producers to pay for additional costs related to analgesia use or apply alternative methods to avoid boar taint. One feasible alternative to castration is immunocastration. The boar receives two injections of a protein compound that induces the production of antibodies against gonadotropin-releasing hormone, which temporarily suppresses testicular development and function, and in turn the production of androsterone and skatole (reviewed in AVMA 2013). While there is an immunocastration product that is approved by the U.S. Food and Drug Administration (FDA) (American Veterinary Medical Association Welfare Division 2013), there is no data available about the use in the U.S. A second alternative would be to raise entire males and slaughter at a prepubertal weight. However, with the U.S. being third largest consumer, and one of the largest exporters of pork in the world (USDA ERS 2019), this could present financial and logistical challenges within the supply chain. Another alternative to current surgical castration practices is to provide analgesic during castration. However, there are currently no analgesic pharmaceuticals approved by the FDA for use in pigs (Bates et al. 2014).

No studies have considered U.S. public perceptions of current castration practices. The public is generally unaware of animal husbandry practices (Alonso et al. 2020) and castration practices. Only 40% of consumers in Flanders, Belgium were aware of the current castration practices in Belgium (Vanhonacker et al. 2009). Brazilian citizens were unaware of the relationship between boar taint in pork and castration (Yunes et al. 2019, Hötzel et al. 2020). Similar results were found for European consumers (Vanhonacker & Verbeke 2011, Heid & Hamm 2013). A pooled survey of consumers from Belgium, France, Germany, and the Netherlands indicated that only 46% of respondents were aware of the existence of boar taint, and 51% were aware of surgical castration (Vanhonacker & Verbeke 2011). German consumers of certified organic products were generally unaware of the connection between intact boars and meat quality, but expressed interested in at least trying a "tainted" product before deciding on future purchasing (Heid & Hamm 2013). With alternatives to surgical castration becoming more feasible for industry application, it is relevant to determine knowledge and perceptions of the public about current swine industry castration practices. In Europe, surgical castration without analgesics was found to be the least accepted method (32%) (Aluwé et al. 2020). There was high acceptance of castration with anaesthesia (85%), immunocastration (71%), and raising intact males (49%) (Aluwé et al. 2020). Consumer pressures could result in changes in legislation or industry standards. Therefore, the objective of this pilot study was to perform an initial investigation into the perceptions and knowledge of the general public and industry stakeholders on current U.S. swine castration practices, through an online survey.

5.3 Methods

All procedures were approved by Virginia Tech Human Research Protection Program Institutional Review Board, protocol #20-404.

5.3.1 Sample

An online Qualtrics survey (SAP, Provo, UT, USA) was distributed in August through September 2020 with the aim to receive responses from experienced swine industry respondents ("industry") and respondents from the general public ("public") at an even ratio. Industry respondents were recruited using Facebook and by direct email to industry stakeholders and university faculty within the authors' network. Facebook posts were non-sponsored and were not distributed in any Facebook groups. Facebook contacts, swine industry contacts, and university staff were invited to share the survey with others, including farm owners, operators, technicians and veterinarians. Public respondents were recruited using Amazon Mechanical Turk (Amazon Web Services, Seattle WA, USA). Public respondents received a monetary compensation through the website, while industry respondents did not receive compensation. All respondents were 18 years or older, and lived in the U.S. Responses were entered anonymously.

We received 129 completed surveys, of which five were omitted because respondents did not live in the U.S., and another five were omitted because respondents failed an attention check question. Survey respondents were categorized based on their experience in the swine industry. Public respondents had no professional swine industry experience, and industry respondents had professional, paid swine industry experience. We included 119 completed surveys in the analysis, 66 from public respondents (55%) and 53 from industry respondents (45%).

5.3.2 Survey instrument

The data presented here originate from a larger survey, with one section presented elsewhere (Neary et al. 2022b). Three out of five sections of the survey consisted of (1) demographic questions, (2) questions on agricultural and swine industry experience, and (3) four questions about perception and knowledge on castration and analgesia procedures. The survey instrument in its entirety is available in Appendix A.

5.3.3 Demographic questions

The first section of the survey included questions about gender (male, female, non-binary, prefer to self-describe, prefer not to say), age category (18-25, 26-35, 36-45, 46-55, 56-65, 66+ years old), home state or territory, community type (city/urban, suburban, rural, other), and highest level of education. Based on home state or territory entries, respondents were categorized into one of five regions in the U.S., including Northeast, Southeast, Midwest, West, and Southwest.

5.3.4 Agriculture and swine industry experience

The second section of the survey included questions about current and past experience with agriculture and swine. These included questions about frequency of visits to an animal production farm in the last twelve months (daily, weekly, monthly, less than monthly but more than once, once, never) and about their work experience with agriculture. After a positive response, questions followed on their experience with animal agriculture and animal species. If respondents had experience with swine, they were asked about the duration of professional work experience (no professional experience, less than 1 year, 1-5 years, 6-10 years, 11+ years). The last question required respondents to indicate their personal experience with castration (none, some, moderate, a lot).

5.3.5 Perception and knowledge of the swine industry castration practices

The main section of the survey contained four questions about the respondents' perceptions and knowledge of common castration procedures within the U.S. swine industry. The first question was whether respondents were aware that castration may occur in the U.S. swine industry prior to the survey, and how many males would undergo the procedure, which was expressed as % of males (never, 1-25% of males, 26-50% of males, 51-75% of males, 76-99% of males, or all males). In the second question, respondents were asked if any type of pain relief (anaesthesia or topical analgesia) was used. If respondents thought pain relief was used, they were asked how many males would receive analgesia (never, 1-25% of castrated males, 26-50% of castrated males, 51-75% of castrated males, 76-99% of castrated males, all castrated males).

5.3.6 Respondent characteristics

Survey respondents' demographics are presented in Table 5.1. Forty-five percent of public respondents had visited an animal production facility at some point in the last year and 15% of public respondents indicated non-professional experience with swine. Fourteen percent of public respondents did not respond to the question about their experience with castration. The majority of industry respondents (85%) visited an animal production facility monthly or more frequently in the last year, while three respondents had not visited one in the last year. Most industry respondents (80%) had moderate or a lot of experience with swine castration, while 11% of industry respondents had no castration experience.

Table 5.1. Summary of public (n=66) and industry (N=53) respondents' responses to demographics questions.

Survey question	Response category	Public Respondents (n)	Industry Respondents (n)
Gender	Male	35	27
	Female	30	26
	Prefer not to say	1	0
Age	18-25	14	12
	26-35	23	19
	36-45	18	15
	46-55	6	4
	56-65	4	3
	66+	1	0
United States region of residency	Northeast	15	4
	Southeast	5	4
	Midwest	21	27
	West	20	16
	Southwest	5	2
Community Type	City/Urban	24	16
	Suburban	29	11
	Rural	13	26
Education Level	Less than a high school degree	0	0
	High school degree or equivalent	4	1
	Some college	14	0
	2-year degree	6	1

	4-year degree	24	26
	Master's degree	10	11
	Professional degree	4	4
	Doctorate	4	10

5.3.7 Statistical analysis

Data residuals were inspected for normal distribution in QQ plots and did not meet the assumptions for normality. Responses for the four questions on knowledge and perception of swine castration were analysed non-parametrically in JMP Pro 15 (SAS Institute Inc., Cary, NC, USA) using the chi-squared test. Response group, demographics (age, gender, region of residency, and community type), swine industry experience (in years) and experience castrating swine were individually analysed for their impact on question responses. Wilcoxon sign-ranked test was used for post-hoc analysis when main effect $P < 0.05$.

5.4 Results

5.4.1 Are you aware that surgical castration may occur in the U.S. swine industry?

Industry experience affected the respondents' awareness of castration practices in the U.S. swine industry ($\chi^2 = 25.758$; df = 1; $P < 0.001$), with 96% of industry respondents indicating they were aware of the practice, compared to 55% of public respondents (Figure 1). Responses were impacted by community type ($\chi^2 = 8.349$; df = 2; $P = 0.015$). Respondents from rural communities (90%) tended to be or were more frequently aware of surgical castration than respondents from suburban (68%; $P = 0.062$) or urban communities (63%; $P = 0.017$; Figure 1). Education level impacted survey responses ($\chi^2 = 14.991$; df = 6; $P = 0.020$). Respondents with a doctorate or a professional degree were more frequently aware of castration practices compared to those with some college education but no degree ($P = 0.010$ and $P = 0.048$ respectively, Figure 1). Respondents' professional experience in the swine industry (expressed in years; $\chi^2 = 28.677$; df = 4; $P < 0.001$) and with performing castration ($\chi^2 = 26.903$; df = 3; $P < 0.001$) impacted their awareness of castration. Respondents with 1-5 years (100%) and 11+ years (100%) of professional swine industry experience were more often aware of the practice compared to those with no professional experience (55%, $P < 0.001$; Figure 1). Respondents with a lot (100%; $P < 0.001$), moderate (93%; $P < 0.001$), and some experience (85%; $P = 0.012$) with swine castration were more often aware of the practice compared to those with no experience with castration (45%; Figure 1). Respondents' age ($\chi^2 = 4.661$; df = 5; $P = 0.459$), gender ($\chi^2 = 3.979$; df = 2; $P = 0.137$), and region of residency ($\chi^2 = 7.121$; df = 5; $P = 0.151$) did not impact their responses to this question.

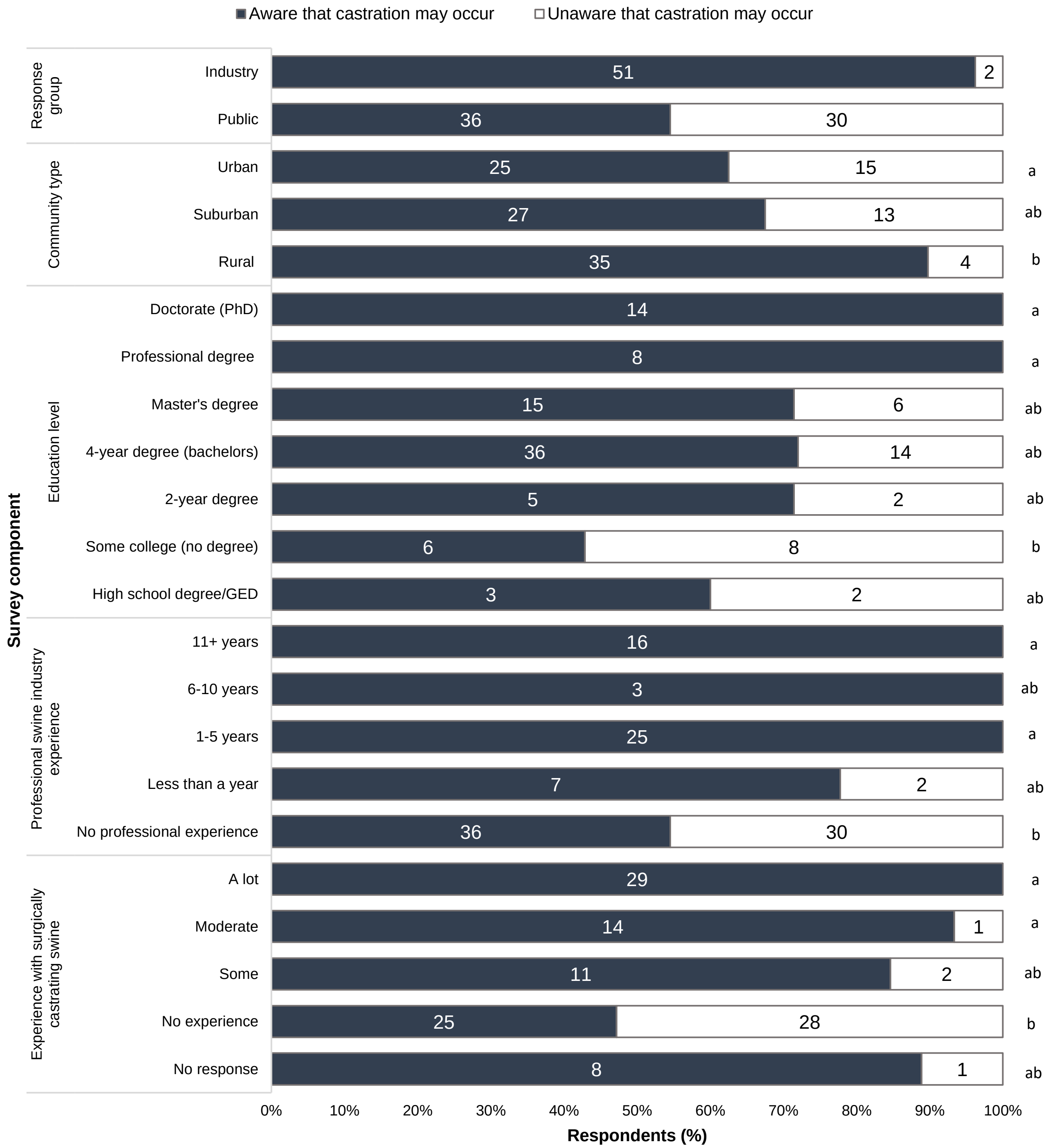

Figure 1. Question: "Are you aware that surgical castration may occur in the U.S. swine industry?" by respondents' response group, level of education, community type, professional swine industry

experience and experience with surgically castrating swine. Respondent n with different superscripts (a-b) indicate a difference at P < 0.05 within survey component.

5.4.2 How often do you think castration occurs within the industry?

More industry respondents (74%) indicated that 76-99% or all males are castrated compared to public respondents (33%; $\chi^2 = 19.168$; df = 1; $P < 0.001$; Figure 2). Community type ($\chi^2 = 15.515$; df = 2; $P < 0.001$), and education level ($\chi^2 = 17.530$; df = 6; $P = 0.008$) impacted the responses to this question (Figure 2). Respondents living in rural communities more frequently indicated that 76-99% or all males are castrated compared to respondents from urban (74% vs. 33%; $P = 0.002$) and suburban communities (48%; $P = 0.010$). Respondents with a doctorate degree more frequently indicated that 76-99% or all males are castrated compared to those with some college but no degree (93% vs 29%; $P = 0.042$). Respondents' professional experience in the swine industry (expressed in years; $\chi^2 = 29.947$; df = 4; $P < 0.001$) and with performing castration ($\chi^2 = 26.407$; df = 3; $P < 0.001$) impacted their perception of castration frequency in the industry. Respondents with 11+ years of experience in the swine industry (94%) more frequently indicated that 76-99% or all males are castrated compared to those with less than a year (44%; $P = 0.006$) and no experience (32%; $P < 0.001$). Those with 1-5 years of experience (69%) also more frequently indicated that 76-99% of males are castrated or that all males are castrated compared to those with no experience (32%; $P = 0.003$; Figure 2). Respondents with a lot of experience castrating swine (90%) more frequently indicated that 76-99% or all males are castrated compared to those with some experience (30%; $P = 0.001$) and those with no experience (30%; $P < 0.001$; Figure 3). Respondents' age ($\chi^2 = 3.264$; df = 5; $P = 0.607$), gender ($\chi^2 = 1.000$; df = 2 $P = 0.659$), and region of residency (7.116; df = 4; $P = 0.130$) did not impact their response.

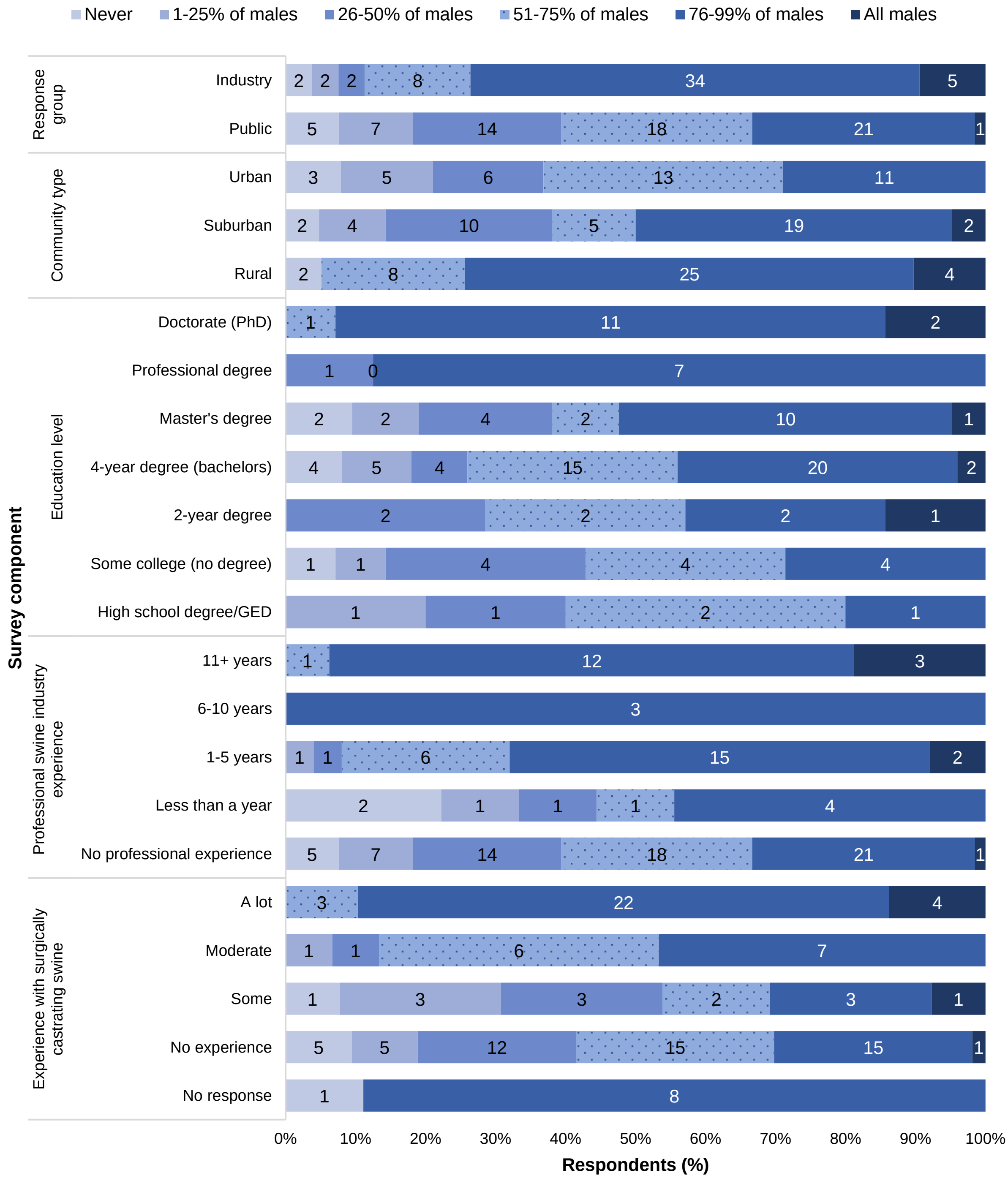

Figure 2. Question "How often do you think castration occurs in the U.S. swine industry?" by respondents' response group, community type, education level, years of professional swine industry experience, and experience with surgically castrating swine.

5.4.3 Do you think any type of pain relief is routinely used during surgical castration in the U.S. swine industry?

Industry experience affected the perception of pain relief use in the U.S. swine industry (χ^2 = 9.9744; df = 1; P = 0.002), with 13% industry respondents indicating 'yes', compared to 39% of public respondents (Figure 3). Region of residency (χ^2 = 14.060; df = 4; P = 0.007) and community type (χ^2 = 7.4060; df = 2; P = 0.025) impacted their responses. Respondents from the Northeast (86%) indicated 'yes' more frequently compared to those from the Southeast (17%; P = 0.003), Southwest (22%; P = 0.047), and the Midwest (25%; P = 0.014; Figure 3). Urban residents (43%) more frequently indicated that piglets would receive pain relief during castration compared to rural residents (15%; P = 0.019; Figure 4).

Respondents' professional experience in the swine industry (in years; χ^2 = 11.3463; df = 4; P = 0.023) and their experience with performing castration impacted their responses (χ^2 = 16.3059; df = 3; P = 0.001). Respondents with no professional experience in the swine industry (28%) indicated 'yes' more frequently than those with 11+ years of experience (0%; P = 0.017; Figure 3). Respondents with no experience (32%; P = 0.026) or moderate experience castrating swine (33%; P < 0.001) more frequently indicated piglets would receive pain relief compared to those with a lot of experience (3%; Figure 3). Respondents' age (χ^2 = 7.931; df = 5; P = 0.160), gender (χ^2 = 1.575; df = 2; P = 0.455), and education level (χ^2 = 8.475; df = 6; P = 0.205) did not impact their responses to this question.

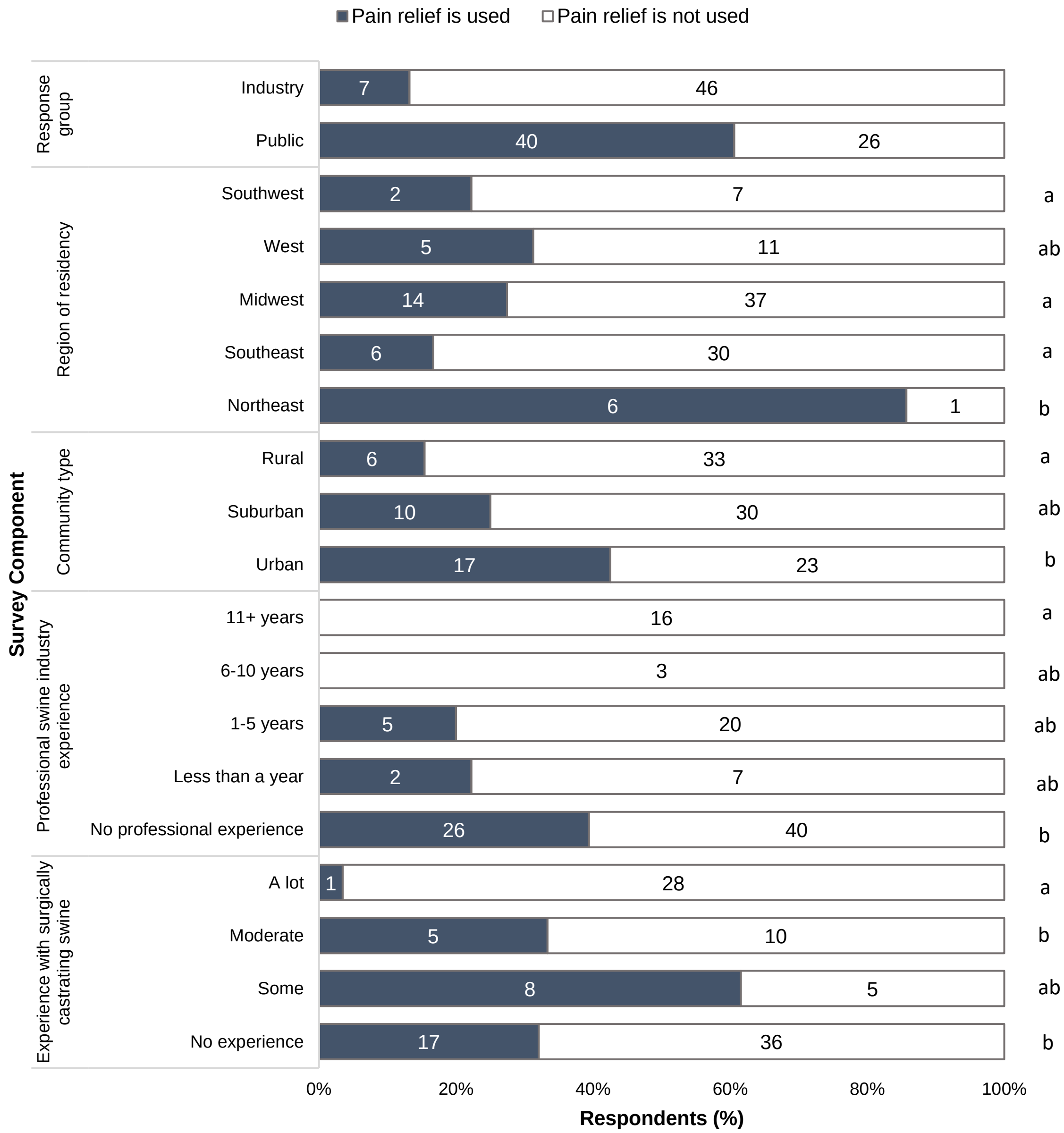

Figure 3. Question: "Do you think any type of pain relief is routinely used during surgical castration in the U.S. swine industry?" by respondents' response group, region of residency, community type, professional swine industry experience, and experience surgically castrating swine. Respondent n with different superscripts (a-b) indicate a difference at $P < 0.05$ within survey component.

5.4.4 If yes, how often would you think pain relief is used for surgical castration in the U.S. swine industry?

The subset of respondents (n=33) that perceived that pain relief is routinely used were asked to quantify the number of males receiving pain relief. Nine percent of respondents thought pain relief was used in 1-25% of castrated males, 42% thought it was used in 26-50% of castrated males, 39% thought it was used in 51-75% of castrated males, and 9% thought it was used in 76-100% of males. Respondents' industry experience ($\chi^2 = 0.410$; df = 1; $P = 0.522$), gender ($\chi^2 = 0.833$; df = 1; $P = 0.361$), education level ($\chi^2 = 7.657$; df = 6; $P = 0.264$), region of residency ($\chi^2 = 2.944$; df = 4, $P = 0.567$), community type ($\chi^2 = 1.173$; df =2; $P = 0.556$), amount of professional swine experience ($\chi^2 = 2.964$; df = 2; $P = 0.139$), and amount of experience castrating swine ($\chi^2 = 3.961$; df = 0.266; $P = 0.189$) did not impact their responses to this question.

5.5 Discussion

Pain associated with surgical castration is a welfare concern for piglets, and all male piglets intended for meat production are surgically castrated without analgesia in the U.S. As alternatives to surgical castration become more feasible to be applied in the industry, and consumer pressures could stimulate the industry to apply alternatives, we need more insight in what the public and industry stakeholders know about current practices. To our knowledge, we are first to gather information on the perceptions of U.S. residents on castration practices in the U.S. swine industry. Survey respondents were representative in terms of gender, but not in terms of age, community type, or level of education, or region when comparing to census data (United States Census Bureau 2019). Survey respondents were younger, more often located in suburban or rural communities, and more highly educated. In terms of region, survey respondents were mostly from the West and

Midwest (30% and 40% respectively), while census data shows that those regions contain 21% of the population. This suggests that in the current study the West and Midwest were overrepresented and respondents from the Southeast (8%) and Southwest (6%) underrepresented (United States Census Bureau 2019). The skew towards the West and the Midwest is due to the concentration of swine production in those regions (NASS 2016). Survey respondents from the Northeast (16%) were sufficiently represented when compared to census data (17%; (United States Census Bureau 2019)). The skewed sample in this study limits the possibility to extrapolate results, especially for representation of the general public, but this pilot study does provide a first insight respondents' perception on U.S. swine industry practices.

In line with expectation, respondents with industry experience and experience with castration were more knowledgeable about the prevalence of surgical castration within the U.S. swine industry. Interestingly, two industry respondents were unaware of the practice. Those individuals had indicated less than a year of professional swine experience and no experience on a sow farm, where castrations occur, explaining their lack of knowledge. Forty-five percent of public respondents were unaware of the practice, which is similar to previous findings in other countries. A minority of participants of a focus group in Norway (% not reported) were aware of routine castration of Norwegian piglets (Fredriksen et al. 2011). Additionally, their survey indicated that 60% of Norwegian consumers were unaware of the practice (Fredriksen et al. 2011). Similarly, a Belgian survey showed that 51% of respondents were unaware that male piglets are castrated (Vanhonacker et al. 2009). The majority (70%) of Brazilian respondents were unaware that surgical castration was the most common method of castration, and 76% of respondents were unaware that all male pigs slaughtered in Brazil are castrated (Yunes et al. 2019). The low number of public respondents knowledgeable about surgical castration is in line with public alienation from livestock production and associated practices (Harper 2001). Low levels of awareness could be associated with low levels of concern. In a meta-analysis of consumer studies, the public generally shows a desire to improve farm animal welfare, regardless of the species or welfare issue being considered (Clark et al. 2017). Therefore, producers and other stakeholders in the industry should be prepared to adjust their surgical castration practices to more welfare-friendly alternatives, in order to maintain their social license (Alonso et al. 2020).

Thirty-one percent of public respondents and 64% of industry respondents indicated that 76-99% of male pigs are castrated, which somewhat aligns with current U.S. industry standards. More precisely, all commercial males intended for meat production are castrated to meet USDA Food Safety and Inspection Services regulations (Rault et al. 2011). With 100% as the correct option, these survey respondents could have considered breeding animals or niche market animals when responding to this question, explaining their lower estimate. One public respondent (2%) and five industry respondents (9%) indicated that all males are castrated, which does align with practice. Sixty-seven percent of public respondents and 27% of industry respondents underestimated the prevalence of castration in the U.S. swine industry. This suggests that in both groups, knowledge about the widespread application of surgical castration is somewhat or mostly lacking.

Analgesia use was overestimated by both response groups. Thirty-nine percent of public respondents and 13% percent of the industry respondents expected that some sort of pain relief is used for piglet castration. In a conventional commercial setting, analgesics are not used for piglet castration, as none are approved for use in swine by the U.S. Food and Drug Administration. It was rather surprising that 13% of industry respondents expected analgesia to be routinely used, especially as 45% indicated they had personal experience with performing surgical castration. Some industry respondents may have said yes because their facility uses analgesia. Although it is not required, producers may take it upon themselves to use off-label analgesia after consultation with a veterinarian. Pain associated with surgical castration (Prunier et al. 2006) warrants the use of analgesia during surgical castration or the use alternative approaches to avoid boar taint. In Brazil, survey respondents showed more support for alternative approaches including analgesia use, immunocastration, or raising entire males, than for surgical castration without analgesia (Hötzel et al. 2020). Whether the public or industry stakeholders support these alternatives in the U.S. is not yet studied.

In our survey, respondents with higher levels of education had a better perception of castration prevalence in the swine industry compared to those with lower levels of education. It should be noted that 10/14 respondents with a PhD degree were part of the industry respondent group, which could have impacted this outcome. However, in line with our findings, highly educated respondents from midwestern states were more knowledgeable about agricultural practices than those with lower levels of education (Frick et al. 1995). In addition, respondents with higher levels of education and higher income rates demonstrated more concern for animal welfare (reviewed by (Alonso et al. 2020)). This greater level of concern could have contributed to the greater levels of knowledge about the industry. Respondents from rural communities were more familiar with castration prevalence and analgesia use compared to respondents from urban communities. With the industrialization of livestock production and the increasing urbanization worldwide (Satterthwaite et al. 2010), the public are further distanced from agricultural practices in rural settings. This could contribute to the respondents' relatively limited knowledge about farming practices.

Our results highlight an opportunity for targeted education on swine industry practices especially for people with lower levels of education and people from urban communities. Although our survey specifically focused on surgical castration in the swine industry, results may indicate a wider trend for a gap in knowledge of the industry in those specific demographics. There is no research about public or industry stakeholder knowledge of swine industry practices, or about the impact of their knowledge and perceptions on product purchasing behaviour in the U.S. Yet, previous work in Europe indicates that consumers that are knowledgeable about surgical castration show aversion. For instance, willingness-to-pay for pork originating from immunocastrated pigs was 12% or 21% higher than for pork from surgically castrated pigs in Germany and Sweden (Lagerkvist et al. 2006, Heid & Hamm 2013). The majority (70%) of surveyed consumers from Germany, the Netherlands and Belgium indicated a preference for immunocastration over surgical castration with analgesia (Vanhonacker & Verbeke 2011). Nearly a third (27%) of Australian survey respondents indicated that they disapproved or strongly disapproved of piglet castration (Grahame et al. 2019). The aversion to surgical castration could be similar in the U.S., and could imply that U.S. consumers would change purchasing behaviour towards pork products originating from pigs that are not surgically castrated. Providing information about surgical castration alternatives to avoid boar taint may affect public attitude (Tuyttens et al. 2011). Therefore, providing that information can be beneficial for public and industry acceptance of surgical castration alternatives, and in turn improve swine welfare.

5.6 Conclusion

In this pilot study, we used an online survey to evaluate the impact of swine industry experience on survey participants' perceptions and knowledge of U.S. swine industry castration practices. Results indicate that mostly public respondents were unaware of castration practices and the (lack of) use of analgesia. Surprisingly, a small number of industry stakeholders also showed gaps in knowledge regarding common practices. We conclude that based on this small survey, information and education on current castration practices and boar taint seems needed, especially for respondents from urban communities and those with lower levels of education. Providing this information can support the public in making informed decisions when purchasing products. Possible subsequent aversion to current practices, as indicated in research from Europe, could lead to consumer pressure towards alternatives to surgical castration preventing boar taint, or use of analgesia as a routine component of surgical castration.

Animal welfare implications

Surgical castration without analgesia is the most common method for preventing the development of boar taint in male piglets in the U.S. swine industry. While there are alternatives, it is important to understand the public's awareness of current castration practices and perceived prevalence of surgical castration and analgesia use in comparison to industry stakeholders. The present pilot study is a first investigation into U.S. public awareness and shows that the public and even some industry personnel are unaware of the process. Further research should focus on a representative sample of the general public and investigate public awareness and acceptance of current castration processes and future alternatives in the U.S.

Declaration of Interest

None.

Acknowledgements

This work was supported by the USDA National Institute of Food and Agriculture, Hatch Multistate project 1024623.

Chapter 6: General Discussion and Conclusion

The research objectives were to 1) investigate the short- and long-term impacts of castration on the affective state in piglets, 2) to evaluate the impact of swine industry experience on survey participants' ability to apply the PGS, 3) to evaluate the ability of all respondents to apply the appropriate PGS scores compared to gold standard ratings by pain experts, and 4) to perform an initial investigation into the perceptions and knowledge of the general public and industry stakeholders on current U.S. swine castration practices.

6.1 Castration and affective states

To our knowledge, this is first attempt to use an attention bias test to investigate the impacts of surgical castration on affective states and the first to apply the test to 1-week old piglets (Chapter 3). There is an impact of surgical castration on the affective state in 1-week old piglets, however any effect is lost by the time piglets reach 12 weeks of age. We also found that analgesic use may reduce anxiety levels, thus improve affective state, in piglets in the 24 hours following castration. This implies that surgical castration does negatively impact all aspects of piglet welfare, including their health, behavior, and affective states. Yet more research is needed to confirm this, as responses did not show a consistent effect on affective states. Furthermore, the use of analgesia could improve piglet welfare during the painful procedure depending on the medication used.

6.2 Recognizing piglet pain using the Piglet Grimace Scale

We are the first to evaluate the impact of swine industry experience on one's ability to apply the Piglet Grimace Scale (Chapter 4). Using an online survey, we presented participants with pictures of surgically castrated and sham-handled piglets. We also tested multiple aspects of internal validity and reliability of the PGS, as applied by non-experts compared to experts. Overall, we conclude that the Piglet Grimace Scale can be reliably applied in a castration context by both industry professionals and members of the public that receive limited training. However, more training than what we provided may be required in order to have scored align better with expert scores. These results imply that there is a tool for industry stakeholders to start assessing pain experience in the short-term. In the long-term, researchers and industry stakeholders should work towards effective pain relief methods, and effective pain relief protocols to apply when pain expression is detected in an industry setting. Applying the PGS could sensitize industry stakeholders to piglet pain and be incentivized to apply analgesics and improve piglet welfare. In the long-term, alternatives to surgical castration should be considered.

6.3 General public knowledge of industry practices

No previous information on U.S. public knowledge of swine industry castration practices was available (Chapter 5). We are the first to show that an overall deficiency of awareness in public respondents of castration and (lack of) analgesics use compared to industry respondents. Surprisingly, some industry respondents were also unaware of common practices. There is an opportunity for education on industry practices, especially for those from urban communities and those with a lower level of education. The implications of providing this education could be increased awareness of common practices among the general public. In turn this could lead to discomfort and aversion related to surgical castration without pain relief. Long-term changes of this painful practice, preferably before possible public outrage about the procedure, could improve piglet welfare and consumer trust in the swine industry.

6.4 Recommendations for further research

Based on our findings from the presented studies, a number of further research suggestions are listed below.

- Investigate biologically relevant positive stimuli for 1-week-old piglets and refine the attention bias test.

The attention bias test discussed in Chapter 3 is modelled after ones used in other studies. However, this was the first time it was used for piglets that were 1 week old. A more biologically relevant positive stimulus may provide more meaningful results for piglets in that age range. There are a limited number of options that can be used because piglets at this age typically are not exposed to solid food. Since there are limited number of studies that have applied the attention bias test in piglet, there is room for improvement with the test. One benefit may be extending the length of the test to ensure a greater response rate. Another approach could include a paired or group-level test approach to reduce fear during the test procedure.

- Investigate effective analgesics for surgical castration in piglets.

Surgical castration is a painful procedure for piglets. The study presented in Chapter 3 provides the first evidence of surgical castration's negative impact on piglets' affective state. We provided the piglets with analgesics to address the acute pain (lidocaine) and chronic pain (flunixin). Even though some of our results showed reduced pain in the treated piglets. Results were not consistent across all measurements. Further research is necessary to determine the most effective analgesic for castration pain relief in piglets.

- Evaluate the proper amount of training needed to obtain more consistent scores between trainees and experts for the Piglet Grimace Scale.

While the results presented in Chapter 4 did suggest that observers can apply the Piglet Grimace Scale after minimal training, regardless of their industry background and experience, they did not score in agreement with experts. Rather they overestimated pain expression in some cases. This indicates that more training is needed in order to avoid over estimation of pain. We know that minimal training is needed to learn the scale, but further research is needed to determine what amount of training is needed to become an expert, and what methods of training are most effective. This will help determine the feasibility of application on a larger commercial scale.

- Investigate the public's understanding of swine industry and evaluate the most effective ways to convey information.

While there is an abundance of research pertaining to the perceptions and expectations of consumers in Europe, little research has been conducted to into the perceptions of U.S. consumers, especially those pertaining to the swine industry. The pilot study presented in Chapter 5 is the first to investigate the knowledge of the public's knowledge on swine castration practices in the industry. Our results indicate a very limited knowledge about castration practices among members of the public, and there are even members of the industry who are unaware of the practice of castration. We need more education on the topic, and likely on the agricultural industry as a whole. We need to evaluate the best way to convey information about the industry. Social media and technology are constantly changing and evolving, thus the way we deliver information needs to evolve as well. More research is needed to evaluate the use of everchanging technology for unbiased, effective approaches to inform the public about industry practices.

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Appendices

Appendix A: Survey Instrument

The following is the survey instrument that was used to collect data described in Chapters 4 and 5. (○ = options to select from)

Introduction and Informed Consent

This survey contains pictures of piglets' faces in 12 scenarios. We will ask you to apply the grimace scoring system (explained in the survey) based on the position of their nose/cheeks, eyes, and ears. The survey ends with some basic demographic questions and questions about your experience with swine. Experience is ***not*** required for this survey. All responses are valuable, no matter the amount of experience!

Identifying pain based on piglet facial expressions can be a useful tool to provide appropriate care to these piglets. A categorical scale to score these pain expressions or grimaces was recently developed. We aim to find out how easy it is for the survey respondents to apply this grimace scale.

The responses will be used to validate the piglet grimace scale and will be published. Your responses will be anonymous.

We are extremely grateful that you are will to participate in this survey and contribute to our data collection.

We thank you for your responses and participation!

Consent to Take Part in a Research Study

Principal Investigator: Leonie Jacobs: jacobsl@vt.edu / 540-231-4735
Other study contact(s): Jessica Neary jessicaneary@vt.edu / 618-830-8916
Detailed Information: The following is more detailed information about this study in addition to the information listed above.

Who can I talk to?

- If you have questions, concerns, or complaints, or think the research has hurt you, talk to the lead for the research team at Virginia Tech, Leonie Jacobs (jacobsl@vt.edu / 540-231-4735).
- This research has been reviewed by the Virginia Tech Institutional Review Board (IRB). You may communicate with them at 540-231-3732 or irb@vt.edu if:
- You have questions about your rights as a research subject
- Your questions, concerns, or complaints are not being answered by the research team
- You cannot reach the research team
- You want to talk to someone besides the research team to provide feedback about this research

How many people will be studied?

- We plan to include about 100 people in this research study.

What happens if I say yes, I want to be in this research?

- The length and duration of procedures: This is a one-time online survey that should take about 20 minutes.

What happens if I say yes, but I change my mind later?

- You can leave the research at any time, for any reason, and it will not be held against you.
- If you decide to leave the research, and do not complete the survey, the answers to the questions that you've already answered may be used for data collection, but the answers cannot be linked to you in any way. All collected information will be anonymized (any identifiable details removed).
- If you decide to leave the research, you may contact the investigator, but you could also simply do nothing. This is an online survey and you can choose not to complete it.

Is there any way being in this study could be bad for me? (Detailed Risks)

- You may find the images of pig facial expressions emotionally disturbing or they may cause emotional discomfort. There are no physical, privacy, legal, social or economic risks associated with this study (see below for examples of each of these risk categories).

What happens to the information collected for the research?

- No identifiable information will be used for research or publication. All survey responses will be stored without identifiable information. We will use your survey responses to evaluate people's ability to recognize pain through piglet facial expressions. These outcomes cannot be linked to you personally in any publication.
- Organizations that may inspect your information include the IRB, Human Research Protection Program, and other authorized representatives of Virginia Tech. Data will be retained for 3 years after completion of the project. Identifiable information will be stored separately from survey responses. The results of this research study may be presented in summary form at conferences, in presentations, reports to the sponsor, academic papers, and as part of a thesis/dissertation.

Can I be removed from the research without my OK?

- The research leader can remove you from the research study without your approval. Possible reasons for removal include incomplete entry, late entry, answers that are deemed unreliable.

Signature Block for Capable Adult

- By clicking "I agree to participate" and proceeding to the next part of the survey, you are indicating your permission to take part in this research.

I agree to be a part of this survey and consent to participating:

  - Yes
  - No

Piglet Grimace Scale Explanation and Training
The grimace scale evaluates ears, cheeks and eyes of piglets as an indicator of experienced pain. The scale includes scores between 0 and 3, with 0 meaning piglets are not in pain, and 3 meaning they are in pain.

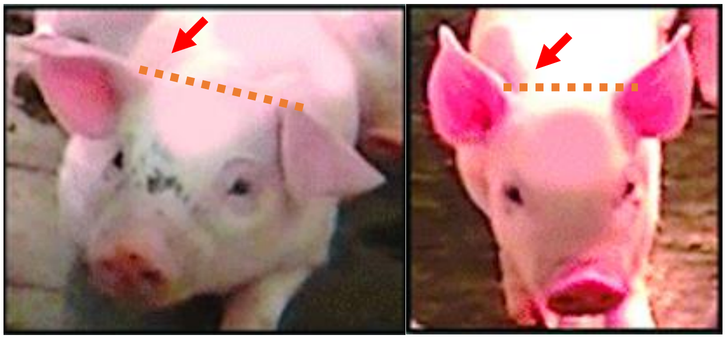
<u>Ear Position</u>

Ears can be in a normal position (score 0), can be positioned backwards (score 1), further back (score 2), or obviously pulled back (score 3).

- Score 0 = no pain (absent)
- Score 1 = some pain (somewhat present)
- Score 2 = moderate pain (moderately present)
- Score 3 = obvious pain (obviously present)

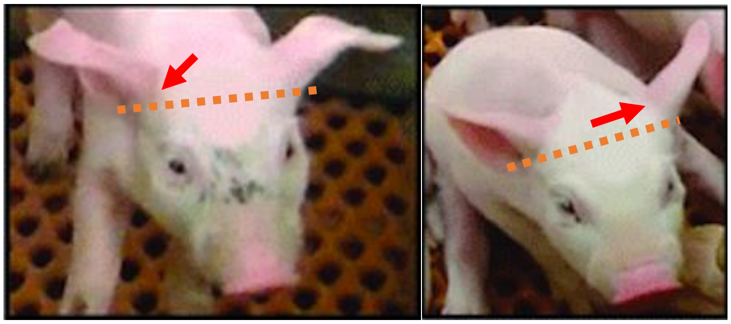
You can determine the ear position by looking at the base of the ear (the point that connects to the piglet's head: see red arrows).

Absent (0): The ears are in baseline position (orange dotted line) and ears are both facing forward, seemly relaxed.

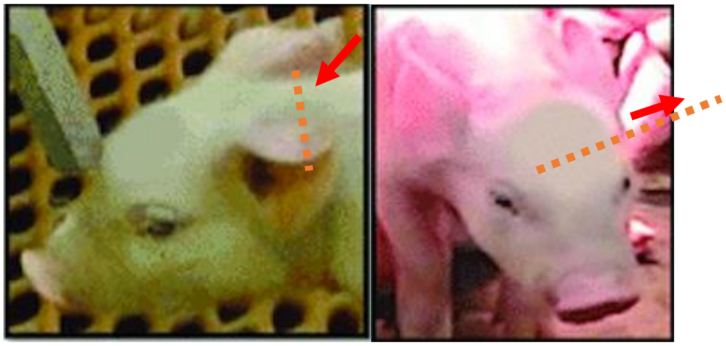


Somewhat present (1): One of the ears is at the baseline and the other is slightly pulled back.

Moderately present (2): Both ears are slightly pulled back, but not pressed against the body.



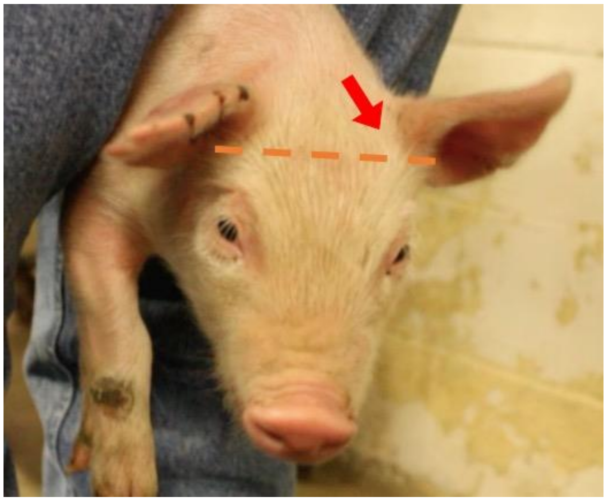
Obviously present (3): Both of the ears are pulled back but are not pressed against the body.



How would you rate this piglet's ear positioning?

- Absent (0)
- Somewhat Present (1)
- Moderately Present (2)
- Obviously Present (3)

We scored this piglet's ear positioning as **somewhat present (1).** One ear is slightly relaxed while the other is pulled back slightly away from the base line.



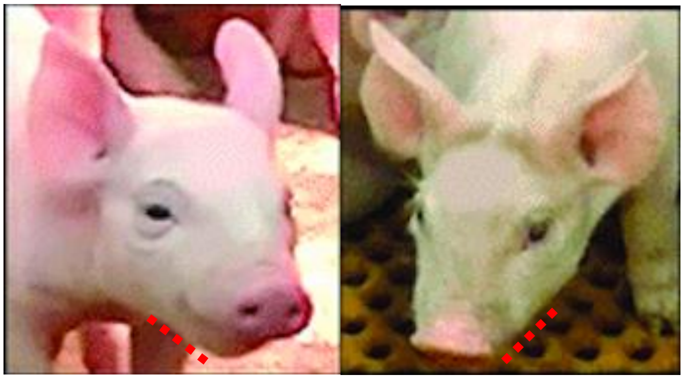
Cheek Tightening

Cheeks can be in a normal position (score 0), can show slight bulging near the snout (score 1), or can show obvious bulging by the snout (score 2).

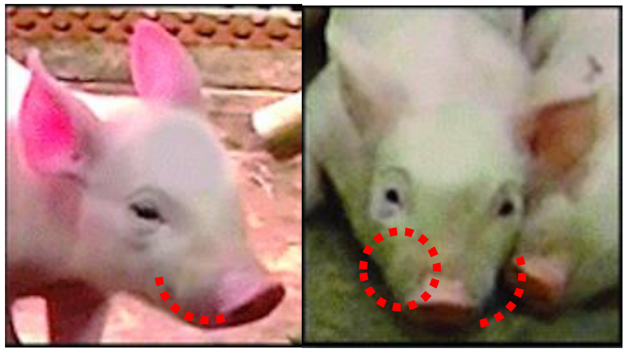
- Score 0 = no pain (absent)
- Score 1 = some pain (somewhat present)
- Score 2 = moderate pain (moderately present)

You can determine the cheek tightening by looking at a profile view of the piglet and looking at the lip line. The bulging is highlighted by the red lines and circles.

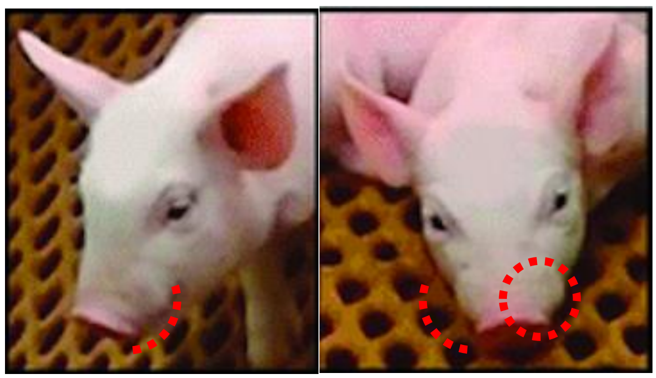
Absent (0): The area right between the nose and cheek is smooth. No bump/bulge is present on the snout (the dotted lines highlight the smooth line and lack of bulge).



Moderately Present (1): There is a slight bulge on the snout (dotted lines and circle).



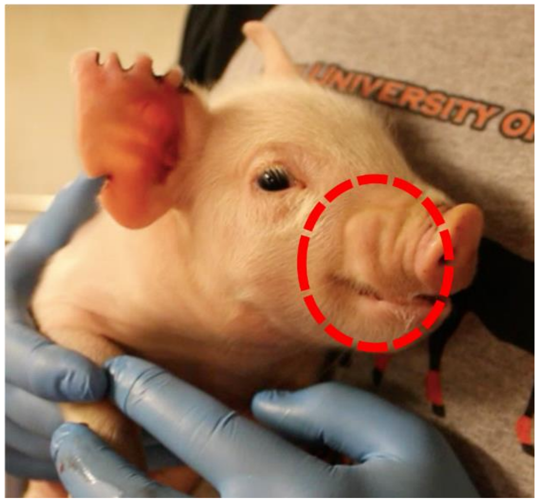
Obviously present (2): There is a clearly noticeable bulge om the snout of the animal (dotted lines and circle).



How would you rate this piglet's cheek tightening?

- Absent (0)
- Moderately Present (1)
- Obviously Present (2)

We scored this piglet's cheek tightening as **obviously present (2).** There is a clear and noticeable bulge on the animal's snout.



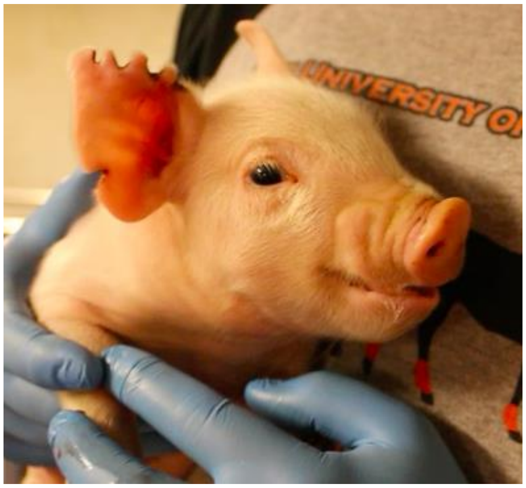
<u>Orbital Tightening</u>

Eyes can be in a normal position, with eyes open similar to human eyes (‘almond shape’) (score 0). When in pain, piglets will squint (orbital tightening) (score 1).

- Score 0 = no pain (absent)
- Score 1 = some pain (squinting/ orbital tightening is present)

Absent (0): Eyes are bright and open(red line)

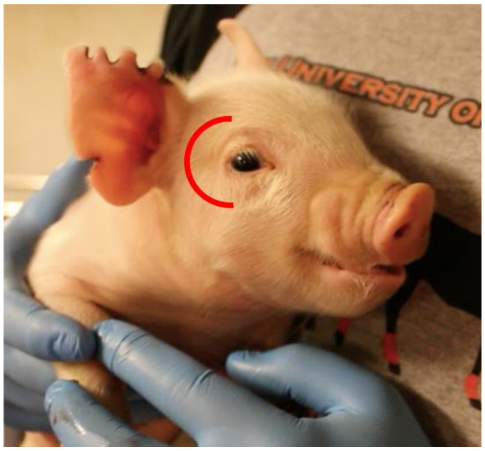
Present (1): Orbital area is narrowed; the eyes are squinting (dotted circle)



How would you rate this piglet’s orbital tightening?

- ○ Absent (0)
- ○ Present (1)

We scored this piglet's orbital tightening as **absent (0)**. The eye is bright and open without 'squinting'.



PGS Category Explanations

For this survey, you will be asked to rate 12 pictures of piglets, evaluating the three different areas explained in the grimace scale. A link to the grimace scale scoring sheet is provided at the bottom of each page for your reference.

We recommend you to keep this reference document open during the survey: *Piglet grimace scale - reference document*

PGS Questions

Scenario 1: How would you rate the following piglet's grimace (ears, cheeks and eyes)? This piglet was castrated right before this photo was taken.

Based on the **ear positioning**, please score the presence of pain (0-3)

- Absent (0)
- Somewhat Present (1)
- Moderately Present (2)
- Obviously Present (3)

Based on **cheek tightening**, please score the presence of pain (0-2)

- Absent (0)
- Moderately present (1)
- Obviously present (2)

Based on the orbital tightening, please score the presence of pain (0-1)

- Absent (0)
- Present (1)

How confident are you with your scores?

Not confident at all | slightly confident | somewhat confident | fairly confident | extremely confident

Scenario 2: How would you rate the following piglet's grimace (ears, cheeks and eyes)? This piglet was castrated right before this photo was taken.

Based on the **ear positioning**, please score the presence of pain (0-3)

- Absent (0)
- Somewhat Present (1)
- Moderately Present (2)
- Obviously Present (3)

Based on **cheek tightening**, please score the presence of pain (0-2)

- Absent (0)
- Moderately present (1)
- Obviously present (2)

Based on the orbital tightening, please score the presence of pain (0-1)

- Absent (0)
- Present (1)

How confident are you with your scores?

Not confident at all | slightly confident | somewhat confident | fairly confident | extremely confident

Scenario 3: How would you rate the following piglet's grimace ears, cheeks and eyes)? This piglet was being castrated while this photo was taken.

Based on the **ear positioning**, please score the presence of pain (0-3)

- Absent (0)
- Somewhat Present (1)
- Moderately Present (2)
- Obviously Present (3)

Based on **cheek tightening**, please score the presence of pain (0-2)

- Absent (0)
- Moderately present (1)
- Obviously present (2)

Based on the orbital tightening, please score the presence of pain (0-1)

- Absent (0)
- Present (1)

How confident are you with your scores?

Not confident at all   slightly confident   somewhat confident   fairly confident   extremely confident

Scenario 4: How would you rate the following piglet's grimace (ears, cheeks and eyes)? This piglet was castrated right before this photo was taken.

Based on the **ear positioning**, please score the presence of pain (0-3)

- Absent (0)
- Somewhat Present (1)
- Moderately Present (2)
- Obviously Present (3)

Based on **cheek tightening**, please score the presence of pain (0-2)

- Absent (0)
- Moderately present (1)
- Obviously present (2)

Based on the orbital tightening, please score the presence of pain (0-1)

- Absent (0)
- Present (1)

How confident are you with your scores?

Not confident at all	slightly confident	somewhat confident	fairly confident	extremely confident

Scenario 5: How would you rate the following piglet's grimace (ears, cheeks and eyes)? This piglet was not castrated.

Based on the **ear positioning**, please score the presence of pain (0-3)

- o Absent (0)
- o Somewhat Present (1)
- o Moderately Present (2)
- o Obviously Present (3)

Based on **cheek tightening**, please score the presence of pain (0-2)

- o Absent (0)
- o Moderately present (1)
- o Obviously present (2)

Based on the orbital tightening, please score the presence of pain (0-1)

- o Absent (0)
- o Present (1)

How confident are you with your scores?

Not confident at all | slightly confident | somewhat confident | fairly confident | extremely confident

Scenario 6: How would you rate the following piglet's grimace (ears, cheeks and eyes)? This piglet was not castrated.

Based on the **ear positioning**, please score the presence of pain (0-3)

- ○ Absent (0)
- ○ Somewhat Present (1)
- ○ Moderately Present (2)
- ○ Obviously Present (3)

Based on **cheek tightening**, please score the presence of pain (0-2)

- ○ Absent (0)
- ○ Moderately present (1)
- ○ Obviously present (2)

Based on the orbital tightening, please score the presence of pain (0-1)

- ○ Absent (0)
- ○ Present (1)

How confident are you with your scores?

Not confident at all | slightly confident | somewhat confident | fairly confident | extremely confident

Scenario 7: How would you rate the following piglet's grimace (ears, cheeks and eyes)? This piglet was castrated right before this photo was taken.

Based on the **ear positioning**, please score the presence of pain (0-3)

- Absent (0)
- Somewhat Present (1)
- Moderately Present (2)
- Obviously Present (3)

Based on **cheek tightening**, please score the presence of pain (0-2)

- Absent (0)
- Moderately present (1)
- Obviously present (2)

Based on the orbital tightening, please score the presence of pain (0-1)

- Absent (0)
- Present (1)

How confident are you with your scores?

Not confident at all   slightly confident   somewhat confident   fairly confident   extremely confident

Scenario 8: How would you rate the following piglet's grimace (ears, cheeks and eyes)? This piglet was not castrated.

Based on the **ear positioning**, please score the presence of pain (0-3)

- Absent (0)
- Somewhat Present (1)
- Moderately Present (2)
- Obviously Present (3)

Based on **cheek tightening**, please score the presence of pain (0-2)

- Absent (0)
- Moderately present (1)
- Obviously present (2)

Based on the orbital tightening, please score the presence of pain (0-1)

- Absent (0)
- Present (1)

How confident are you with your scores?

Not confident at all | slightly confident | somewhat confident | fairly confident | extremely confident

Scenario 9: How would you rate the following piglet's grimace (ears, cheeks and eyes)? This piglet was being castrated while this photo was taken.

Based on the **ear positioning**, please score the presence of pain (0-3)

- Absent (0)
- Somewhat Present (1)
- Moderately Present (2)
- Obviously Present (3)

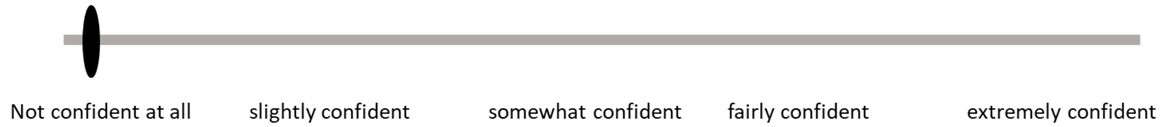
Based on **cheek tightening**, please score the presence of pain (0-2)

- Absent (0)
- Moderately present (1)
- Obviously present (2)

Based on the orbital tightening, please score the presence of pain (0-1)

- Absent (0)
- Present (1)

How confident are you with your scores?



Demographic Questions

What is your gender?

- Female
- Male
- Non-binary/ third gender
- Prefer to self-describe:__________
- Prefer not to say

What is your age range?

- 18-25
- 26-35
- 36-45
- 46-55
- 56-65
- 65+

What state do you currently live in? :________________________________

What type of community do you currently live in?

- City/urban
- Suburban
- Rural
- Other (self-describe):________________________________

What is your highest level of education? (If you're currently enrolled in school, please indicate the highest degree you have received)

- Less than high school
- High school diploma or equivalent (GED)
- Some College (no degree)
- 2-year degree
- 4-year degree (bachelors)
- Master's degree
- Professional degree (for example MD, DMV)
- Doctorate degree
- Other: (self-describe)

Do you consume meat (beef, pork, chicken, fish, other)?

- Yes
- No

Do you have pets?

- Yes
- No

Agricultural and swine experience

How often have you visited an animal production farm in the last 12 month?

- Daily
- Weekly
- Monthly
- Less than monthly but more than once
- Bi-annually
- Once
- Never

Do you have any experience with agriculture?

- Yes
- No

If yes, do you have experience with any type of animal agriculture?

- Yes
- No

Sub Question: What species do you have access to on the farm you visit?

- Pigs
- Beef cattle
- Dairy cattle
- Poultry
- Sheep
- Goats
- Horses
- Other:______________________________

Do you have any professional work experience with swine and the swine industry?

- No professional experience
- Less than 1 year
- 1-5 years
- 6-10 years
- 11+ years

Please indicate the type(s) of operations you have experience with and the largest number of head.

- Commercial sow farm: _(# of head)_
- Commercial nursery/finisher: _(# of head)_
- Non-commercial farm: _(# of head)_

Please provide a short description of the non-commercial swine farm you have experience with:______________________________

How much experience with performing surgical castration do you have in your lifetime?

- No experience performing surgical castration
- Some experience (performed surgical castration 1-10 times in total)
- Moderate experience (performed surgical castration 10-50 times in total)

- A lot of experience (performed surgical castration more than 50 times in total)

Industry knowledge and perceptions

For this section of the survey, we are trying to gather some information on the general perspective of the swine industry and the potential use of surgical castration.

Prior to this survey, were you aware that surgical castration may occur in the swine industry?

- Yes
- No

How often do you think castration occurs in the US swine industry?

- Never
- 1-25% of the males are castrated
- 26-50% of males are castrated
- 51-75% of males are castrated
- 76-99% of males are castrated
- All males are castrated

Do you think any type of pain relief is routinely used during surgical castration in the US swine industry (anesthesia, topical analgesia)?

- Yes
- No

  If yes: How often would you think pain relief is used for surgical castration in the US swine industry?

  - on 1%-25% of the castrated males
  - on 26%-50% of the castrated males
  - on 51-75% of the castrated males
  - on 76-100% of the castrated males